厚生科学研究費補助金(長寿科学総合研究事業)
分担研究報告書

転倒歴と骨密度に関する研究

分担研究者　新野　直明　桜美林大学大学院教授

研究要旨　地域中高齢者を対象に、末梢型定量コンピュータ断層装置(pQCT)、dual energy X-ray absorptiomety(DXA)により測定した骨密度がその後の転倒歴に関係するかを縦断的に検討した。性、年齢別に、握力、主観的健康感、転倒既往を考慮しながら分析したところ、60 歳以上女性において、骨折の危険性が高いと考えられる骨密度の低い人に将来転倒する可能性が高い傾向があった。

A. 研究目的

高齢者の転倒、特に骨折につながる転倒は、寝たきりの主要な原因となる。骨折しやすい転倒を規定する要因はいまだ定かではないが、骨の強度の低下すなわち骨量減少が何らかの関係を持っていることは確かと思われる。したがって、骨折、骨量減少予防を念頭におきながら、転倒と骨密度の関係を検討することは、骨折しやすい転倒の減少につながる貴重な研究になると期待される。そこで、本研究では、地域中高齢者を対象に転倒経験の有無と骨密度の関係に着目した。昨年度は、両者の関係を横断的に分析し、60 歳以上女性において、骨密度が低い場合、過去 1 年間に転倒既往がある傾向が認められた。今年度は骨密度がその後の転倒の有無に関係するかを縦断的に検討した。

B.研究方法

1)対象

長寿医療研究センター疫学研究部の主催する老化に関する長期縦断疫学調査(NILS-LSA)の第一回調査とほぼ 2 年後に実施された第二回調査に参加し、事項の分析項目について情報の得られた 1546 名(60 歳以上 694 名:男性 369 名、女性 325 名、60 歳未満 852 人:男性 438 名, 女性 414 名)である。

2)分析項目

①第一回調査 (1st) における転倒歴：自記式の調査票により、過去1年間の転倒経験の有無を調べた。

②第一回調査 (1st) における骨密度：末梢型定量コンピュータ断層装置 (pQCT) にて非利き手側橈骨を測定した [D50 (海綿骨) 、D100 (海綿骨＋皮質骨) 、P100 (皮質骨)] 。さらに、dual energy X-ray absorptiomety (DXA) により 5 カ所 [全身骨、腰椎、大腿骨頸部、大転

子部、Ward 三角］を測定した。本研究では、平均値未満を低群、平均値以上を高群とした。

③第一回調査（1st）における主観的健康感：自記式の調査票により、主観的健康感の程度を調べた。低群（非常に悪い・悪い）、高群（非常に良い・良い・普通）の 2 群とした。

④第一回調査（1st）における握力：両腕の握力を測定。左右の平均値を算出して個人の測定値とした。さらに、性、年齢（60 歳未満と 60 歳以上）の組み合わせ別に平均値を求め、平均値未満を低群、平均値以上を高群とした。

⑤第二回調査（2nd）における転倒歴

3)分析

第 1 回調査における骨密度と第 2 回調査における転倒歴の関係を、過去の研究で転倒の関連要因とされることの多い主観的健康感、握力を考慮しながら [1]、性、年齢別(60 歳未満と 60 歳以上)に分析した。具体的には、2nd 転倒歴を目的変数、1st 骨密度、1st 主観的健康感、1st 握力、1st 転倒歴を説明変数とした多重ロジスティック回帰分析を、性、年齢別に実施した。

(倫理面への配慮)

NILS-LSA では、参加者に対し口頭と文章による調査説明を行い、調査参加とデータ使用に関する同意書に承諾の署名を得られたに人にのみ調査を実施している。なお、NILS-LSA は、国立長寿医療センターにおいて倫理委員会により承認を受けている。

C.研究結果

1)2nd 転倒歴

今回の対象者 1546 名(60 歳以上 694 名:男性 369 名、女性 325 名、60 歳未満 852 人:男性 438 名, 女性 414 名)において、2 回目調査で転倒を経験した人の割合は、60 歳以上が 22.0%(男性 20.9%、女性 23.3%)、60 歳未満が 16.1%(男性 12.6%、女性 19.6%)であった。

2)骨密度と転倒歴の関係

8種類の 1st 骨密度の各々と 2nd 転倒歴の関係を検討した多重ロジスティック回帰分析の結果を、性、年齢別に表に示した(表 1:60 歳未男性、表 2:60 歳未満女性、表 3:60 歳以上男性、表 4:60 歳以上女性)。男性では、60 歳未満の全身骨を除いて、骨密度とその後の転倒歴には有意な関係は見られなかった。女性では、60 歳未満で骨密度の高い人に転倒が起きる傾向があった(8 個の骨密度測定値の中で 3 個の測定値が有意(P<0.1)に関係)。60 歳以上では、骨密度が低い場合に転倒発生する危険性が高い結果であった(8 個の骨密度測定値の中で 4 個のが有意に関係)。

D.考察

昨年度の研究では、60 歳以上の女性において、転倒歴のある人に骨密度が低い傾向が見られた。しかし、横断的な分析であったために、骨密度の低い高齢女性に転倒が発生したのか、それとも、転倒に伴う行動量の低下などにより骨密

度が低下したのかという点に言及できなかった。また、過去の研究で、骨量と転倒の関係に影響するとされた筋力[2]のコントロールができなかった。そこで、今年度は、筋力など関連要因を考慮しながら、骨密度がその後の転倒発生に関連するかを縦断的に検討した。その結果、60歳以上の女性において、骨密度の低いことが、将来の転倒発生の危険性を高める可能性が示された。高齢女性において、骨折の危険性が高い低骨密度者の転倒危険性が高い傾向にあることは、転倒予防、骨折予防を考える際に十分に留意しなければならない事項と考えられる。

60歳未満の女性では、骨密度の高い女性の方が転倒する危険性の高い傾向があった。これは骨密度の低いことが転倒を予防する方向に働くというよりは、骨が丈夫で歩行を始めとする運動量の多い人が転倒する機会が多いということではないかと思われるが、この点については、運動量などを考慮した更なる検討が必要であろう。

なお、今回の研究目的とは直接関係しないが、いずれの性年齢においても、過去の転倒歴はその後の転倒発生と強く関係していた。近い過去の転倒既往が、転倒発生のハイリスク者を見つける場合に有効であると言われるが[3]、今回の結果もそれを裏付けるものであった。

E.結論

NILS-LSAに参加した地域中高齢者を対象に、骨密度がその後の転倒歴に関係するかを縦断的に検討した。性、年齢別に、握力、主観的健康感、転倒既往を考慮しながら分析したところ、60歳以上女性において、骨折の危険性が高いと考えられる骨密度の低い人に将来転倒する可能性が高い傾向が認められた。

参考文献

1) Tideiksaar R.: Falling in old age: Its prevention and management, 2nd Ed. Springer, New York, 1997.
2) 杉森裕樹：地域の高齢者における転倒と骨量の関連性の検討、平成11年度厚生労働省長寿科学総合研究「地域の高齢者における転倒・骨折の発生と予防に関する疫学的研究」報告書（主任研究者：新野直明）.31-38. 1999. 3) 新野直明：高齢者の転倒とその対応.治療, 283,80-83,2001.

F.健康危険情報

特になし

G.研究発表

1.論文発表

西田裕紀子・新野直明・小笠原仁美・安藤富士子・下方浩史　：地域在住高年者の転倒恐怖感に関連する要因の検討、日本未病システム学会雑誌、2004、10、97-99.

新野直明：高齢者の転倒防止.　福地義之助（編）高齢者ケアマニュアル：58-61、2004、照林社

新野直明：転倒リスクの多因子評価、Geriatr. Med、2005、43、61-65.

Harada A, Matsui Y, Mizuno M, Tokuda H, Niino N, Ohta T: Japanese

orthopedists' interest in prevention of fractures in the elderly from falls, Osteoporos Int. 2004, 15, 560-566

2.学会発表

西田裕紀子、新野直明、他:地域在住高年者における転倒恐怖感の要因に関する縦断的検討. 第 11 回日本未病システム学会.さいたま、2005 年 1 月

H.知的財産権の出願・登録状況(予定を含む)

特になし

表1 ロジスティック回帰分析結果(60歳未満男性)
結果変数:転倒歴2nd[無=0,有=1]

	Odds ratio	95%CI
D50(低)	0.66	0.36-1.20
握力(低)	1.47	0.81-2.69
主観的健康感(不良)	1.76	0.70-4.54
1st転倒歴(有)	7.33 ***	3.61-14.89
D100(低)	0.76	0.42-1.38
握力(低)	1.42	0.78-2.59
主観的健康感(不良)	1.78	0.70-4.50
1st転倒歴(有)	7.20 ***	3.56-14.57
P100(低)	0.63	0.34-1.18
握力(低)	1.37	0.76-2.51
主観的健康感(不良)	1.90	0.75-4.81
1st転倒歴(有)	7.49 ***	3.68-15.25
全身骨(低)	0.53 *	0.29-0.98
握力(低)	1.49	0.81-2.73
主観的健康感(不良)	1.69	0.66-4.32
1st転倒歴(有)	7.57 ***	3.69-15.52
腰椎(低)	0.60	0.33-1.10
握力(低)	1.51	0.83-2.77
主観的健康感(不良)	1.77	0.69-4.52
1st転倒歴(有)	7.41 ***	3.64-15.11
大腿骨頸部(低)	1.57	0.84-2.90
握力(低)	1.34	0.73-2.45
主観的健康感(不良)	1.73	0.68-4.37
1st転倒歴(有)	7.08 ***	3.50-14.34
大転子部(低)	1.24	0.67-2.31
握力(低)	1.37	0.75-2.51
主観的健康感(不良)	1.82	0.72-4.59
1st転倒歴(有)	7.24 ***	3.58-14.64
Ward三角(低)	1.33	0.72-2.44
握力(低)	1.37	0.75-2.51
主観的健康感(不良)	1.78	0.71-4.50
1st転倒歴(有)	7.30 ***	3.61-14.79

***p<.001

表2 ロジスティック回帰分析結果(60歳未満女性)
結果変数:転倒歴2nd[無=0,有=1]

	Odds ratio	95%CI
D50(低)	0.6 †	0.36-1.00
握力(低)	1.24	0.75-2.05
主観的健康感(不良)	.89	0.34-8.69
1st転倒歴(有)	4.96 ***	2.83-8.68
D100(低)	0.63 †	0.37-1.05
握力(低)	1.21	0.73-1.99
主観的健康感(不良)	.89	0.34-2.30
1st転倒歴(有)	5.08 ***	2.89-8.92
P100(低)	0.61 †	0.36-1.02
握力(低)	1.19	0.72-1.97
主観的健康感(不良)	.91	0.35-2.34
1st転倒歴(有)	5.01 ***	2.86-8.78
全身骨(低)	1.13	0.67-1.89
握力(低)	1.13	0.67-1.89
主観的健康感(不良)	.87	0.33-2.28
1st転倒歴(有)	4.81 ***	2.76-8.39
腰椎(低)	0.78	0.46-1.31
握力(低)	1.23	0.74-2.06
主観的健康感(不良)	.91	0.35-2.36
1st転倒歴(有)	4.73 ***	2.72-8.22
大腿骨頸部(低)	1	0.60-1.66
握力(低)	1.17	0.70-1.95
主観的健康感(不良)	.88	0.34-2.29
1st転倒歴(有)	4.75 ***	2.73-8.27
大転子部(低)	0.81	0.49-1.35
握力(低)	1.21	0.73-2.01
主観的健康感(不良)	.87	0.33-2.26
1st転倒歴(有)	4.79 ***	2.75-8.35
Ward三角(低)	0.79	0.47-1.31
握力(低)	1.23	0.74-2.05
主観的健康感(不良)	.90	0.34-2.36
1st転倒歴(有)	4.78 ***	2.75-8.32

†p<.10 ***p<.001

表3 ロジスティック回帰分析結果(60歳以上男性)
結果変数:転倒歴2nd[無=0,有=1]

	Odds ratio	95%CI
D50(低)	0.88	0.56-1.41
握力(低)	1.33	0.84-2.11
主観的健康感(不良)	1.75 †	0.90-3.40
1st転倒歴(有)	4.68 ***	2.73-8.03
D100(低)	1.06	0.67-1.69
握力(低)	1.28	0.81-2.04
主観的健康感(不良)	1.79 †	0.92-3.47
1st転倒歴(有)	4.63 ***	2.70-7.94
P100(低)	0.87	0.55-1.38
握力(低)	1.32	0.84-2.09
主観的健康感(不良)	1.76 †	0.91-3.41
1st転倒歴(有)	4.62 ***	2.69-7.91
全身骨(低)	1.14	0.71-1.81
握力(低)	1.26	0.79-2.01
主観的健康感(不良)	1.81 †	0.93-3.52
1st転倒歴(有)	4.66	2.72-7.99
腰椎(低)	0.85	0.54-1.34
握力(低)	1.31	0.83-2.07
主観的健康感(不良)	1.75 †	0.90-3.39
1st転倒歴(有)	4.63 ***	2.70-7.94
大腿骨頸部(低)	0.89	0.56-1.41
握力(低)	1.38	0.84-2.12
主観的健康感(不良)	1.78 †	0.92-3.44
1st転倒歴(有)	4.64 ***	2.71-7.96
大転子部(低)	0.91	0.57-1.44
握力(低)	1.32	0.83-2.09
主観的健康感(不良)	1.78 †	0.92-3.44
1st転倒歴(有)	4.61 ***	2.69-7.91
Ward三角(低)	1.28	0.79-2.06
握力(低)	1.21	0.75-1.95
主観的健康感(不良)	1.81 †	0.94-3.51
1st転倒歴(有)	4.73 ***	2.75-8.13

†p<.10 ***p<.001

表4 ロジスティック回帰分析結果(60歳以上女性)
結果変数:転倒歴2nd[無=0,有=1]

	Odds ratio	95%CI
D50(低)	1.53 †	0.93-2.53
握力(低)	.94	0.58-1.54
主観的健康感(不良)	1.24	0.61-2.53
1st転倒歴(有)	4.55 ***	2.73-7.59
D100(低)	1.53 †	0.93-2.50
握力(低)	.97	0.59-1.57
主観的健康感(不良)	1.16	0.57-2.36
1st転倒歴(有)	4.49 ***	2.69-7.49
P100(低)	1.34	0.83-2.16
握力(低)	1.01	0.62-1.63
主観的健康感(不良)	1.14	0.56-2.33
1st転倒歴(有)	4.52 ***	2.71-7.53
全身骨(低)	1.55 †	0.95-2.54
握力(低)	.94	0.57-1.53
主観的健康感(不良)	1.21	0.60-2.46
1st転倒歴(有)	4.56 ***	2.73-7.61
腰椎(低)	.99	0.61-1.61
握力(低)	1.02	0.63-1.66
主観的健康感(不良)	1.17	0.57-2.37
1st転倒歴(有)	4.64 ***	2.79-7.73
大腿骨頸部(低)	1.10	0.68-1.79
握力(低)	1.01	0.62-1.63
主観的健康感(不良)	1.16	0.57-2.36
1st転倒歴(有)	4.63 ***	2.78-7.70
大転子部(低)	1.03	0.63-1.67
握力(低)	1.01	0.62-1.66
主観的健康感(不良)	1.17	0.57-2.37
1st転倒歴(有)	4.63 ***	2.78-7.72
Ward三角(低)	2.04 **	1.24-3.34
握力(低)	.92	0.56-1.50
主観的健康感(不良)	1.19	0.58-2.41
1st転倒歴(有)	4.61 ***	2.75-7.72

†p<.10 **p<.05 ***p<.001

Ⅲ. 研究成果の刊行に関する一覧表

雑 誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
YamadaY, Ando F, Niino N, Shimokata H	Association of a polymorphism of the matrix metalloproteinase-9 gene with bone mineral density in Japanese men	Metabolism	53	135-137	2004
Ohta S, Ohsawa I, Kamino K, Ando F, Shimokata H	Mitochondial ALDH2 Deficiency as an Oxidative Stress	Ann NY Acad Sci	1011	36-44	2004
西田裕紀子、新野直明、小笠原仁美、安藤富士子、下方浩史	地域在住高年者の転倒恐怖感に関連する要因の検討	日本未病システム学会雑誌	10	97-99	2004
Koda M, Ando F, Niino N, Shimokata H, Miyashita K, Funakoshi A	Association of Colecystokinin 1 Receptor and β3-Adrenergic Receptor Polymorphisms with Midlife Weight Gain	Obec Res	8	1212-1216	2004
Suzuki Y, Fujisawa M, Ando F, Niino N, Ohsawa I, Shimokata H, Ohta S.	Alcohol Dehydrogenase 2 Variant is Associated with Cerebral Infarction, Lacunae, LDL-Cholesterol and Hypertension in Community-dwelling Japanese Men	Neurology	63	1711-1713	2004
Shimokata H, Ando F, Fukukawa Y	Interactions between health and psychological changes in Japanese - the NILS-LSA	Geriatrics and Gerontology International	4	S289-291	2004
下方浩史	高齢者の健康と栄養	人間の医学	40	132-141	2004
下方浩史	高齢者医療と栄養管理	治療学	38	829	2004
下方浩史	高齢者の健康と栄養～栄養の管理と評価～	若さの栄養学	122	2-10	2004
今井具子、安藤富士子、下方浩史	高齢者におけるサプリメントの利用状況	臨床栄養	104	769-772	2004
Tanaka M, Shimokata H et al.	Mitochondrial Genome Variation in Eastern Asia and the Peopling of Japan	Genome Res	14	1832-1850	2004
Harada A, Niino N, et al	Japanese orthopedists' interest in prevention of fractures in the elderly from falls	Osteoporos Int	15	560-566	2004
新野直明	転倒リスクの多因子評価	Geriatr Med	43	61-65	2005
Yamada Y, Ando F, Niino N, Shimokata H	Association of polymorphisms of the androgen receptor and klotho genes with bone mineral density in Japanese women	J Mol Med	83	50-57	2005

(次ページへ)

(前ページより続く)

Shimokata H, Ando F, Niino N, Miyasaka K, Funakoshi A	Cholecystokinin A receptor gene promoter polymorphism and intelligence	Ann Epidemiol	15	196-201	2005
Yamada Y, Ando F, Niino N, Shimokata H	Association of a -1997G→T polymorphism of collagen Ia1 gene with bone mineral density	Hum Biol	77	27-36	2005
Yamada Y, Ando F, Shimokata H	Association of polymorphisms in CYP17, MTP, and VLDLR with bone mineral density in community-dwelling Japanese women and men	Genomics	in press		
小笠原仁美, 新野直明, 安藤富士子, 下方浩史	中年期地域住民における転倒の発生状況	保健の科学	in press		

書 籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
下方浩史	高齢者の栄養と食生活	沖増 哲	ウエルネス公衆栄養学 第5版	医歯薬出版	東京	2004	195-206
下方浩史	栄養疫学の考え方と方法	沖増 哲	ウエルネス公衆栄養学 第5版	医歯薬出版	東京	2004	35-47
新野直明	高齢者の転倒防止	福地義之助	高齢者ケアマニュアル	照林社	東京	2004	58-61
安藤富士子	高齢者の看護・介護	飯島節、鳥羽研二	老年医学テキスト	南江堂	東京	2005	in press

Ⅳ. 研究成果の刊行物・別刷

Metabolism
Clinical and Experimental

VOL 53, NO 2 FEBRUARY 2004

PRELIMINARY REPORT

Association of a Polymorphism of the Matrix Metalloproteinase–9 Gene With Bone Mineral Density in Japanese Men

Yoshiji Yamada, Fujiko Ando, Naoakira Niino, and Hiroshi Shimokata

Matrix metalloproteinase–9 (MMP-9) is implicated in bone remodeling. A −1562C→T polymorphism in the promoter of the MMP-9 gene (*MMP9*) has been shown to influence gene transcription. The possible relation of this polymorphism to bone mineral density (BMD) was examined in 1,114 Japanese men and 1,087 women. BMD for the total body, lumbar spine, femoral neck, trochanter, or Ward's triangle was significantly lower in the combined group of men with the *CT* or *TT* genotypes or in men with the *CT* genotype than in those with the *CC* genotype. No significant differences in BMD among *MMP9* genotypes were observed in premenopausal or postmenopausal women. The −1562C→T polymorphism of *MMP9* was thus associated with BMD in Japanese men.

MATRIX metalloproteinase–9 (MMP-9) is produced by osteoclasts in human bone and is implicated both in bone resorption,[1-3] as well as in bone formation.[4] A C→T polymorphism at position −1562 in the promoter of the MMP-9 gene (*MMP9*) has been shown to affect transcriptional activity, with the T allele being associated with increased gene transcription.[5] We have now examined whether this polymorphism is associated with bone mineral density (BMD) in a population-based study.

MATERIALS AND METHODS

The National Institute for Longevity Sciences–Longitudinal Study of Aging is a population-based prospective cohort study of aging and age-related diseases.[6] We examined the possible association of BMD at various sites with the −1562C→T polymorphism of *MMP9* in 1,114 Japanese men and 1087 women. The study protocol was approved by the Committee on the Ethics of Human Research of the National Institute for Longevity Sciences, and written informed consent was obtained from each subject. BMD for the total body, lumbar spine (L2 to L4), right femoral neck, right trochanter, and right Ward's triangle was measured by dual-energy x-ray absorptiometry.

Genotypes were determined with a fluorescence-based allele-specific DNA primer assay system. The polymorphic region of *MMP9* was amplified by the polymerase chain reaction with allele-specific sense primers labeled at the 5′ end with either fluorescein isothiocyanate (5′-CCGAGTAGCTGGTATTATAGGXAT-3′) or Texas red (5′-CGAGTAGCTGGTATTATAGGXGT-3′) and with an antisense primer labeled at the 5′ end with biotin (5′-AAACCAGCCTGGTCAACGTA-3′). The reaction mixtures (25 μL) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/L of each deoxynucleoside triphosphate, 4.5 mmol/L $MgCl_2$, and 1 U of Taq DNA polymerase in buffer. The amplification protocol comprised initial denaturation at 95°C for 5 minutes, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 66.5°C for 30 seconds, extension at 68°C for 30 seconds, and a final extension at 68°C for 2 minutes. 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 supernatants from each well were transferred to the wells of a 96-well plate containing 0.01 mol/L NaOH and then measured for fluorescence with a microplate reader.

Quantitative data were compared among 3 groups by 1-way analysis of variance and the Tukey-Kramer post hoc test, and between 2 groups by the unpaired Student's *t* test. BMD values were analyzed with

From the Department of Gene Therapy, Gifu International Institute of Biotechnology, Kakamigahara, Gifu, Japan; and the Department of Epidemiology, National Institute for Longevity Sciences, Obu, Aichi, Japan.

Submitted March 21, 2003; accepted September 27, 2003.

Supported in part by Research Grants for Longevity Sciences (12C-01) (to Y.Y. and H.S.) and by Health and Labor Sciences Research Grants for Comprehensive Research on Aging and Health (H15-Chojyu-014) (to Y.Y., F.A., N.N., and H.S.) from the Ministry of Health, Labor, and Welfare of Japan.

Address reprint requests to Yoshiji Yamada, MD, PhD, FAHA, Department of Gene Therapy, Gifu International Institute of Biotechnology, 1-1 Naka-Fudogaoka, Kakamigahara, Gifu 504-0838, Japan.

0026-0495/04/5302-0005$30.00/0
doi:10.1016/j.metabol.2003.09.003

Table 1. BMD and Other Characteristics of Men (n = 1,114) or of Premenopausal (n = 279) or Postmenopausal (n = 808) Women According to the −1562C→T Genotype of *MMP9*

Characteristic	*CC*	*CT*	*TT*	*CT* + *TT*
Men				
No. (%)	794 (71.3)	280 (25.1)	40 (3.6)	320 (28.7)
Age (yr)	59.0 ± 0.4	59.9 ± 0.7	58.7 ± 1.7	59.7 ± 0.6
BMI (kg/m²)	22.9 ± 0.1	22.8 ± 0.2	23.1 ± 0.4	22.9 ± 0.2
Fracture (%)	201 (25.3)	76 (27.1)	11 (27.5)	87 (27.2)
BMD values (g/cm²)				
Total body	1.090 ± 0.003	1.076 ± 0.006	1.081 ± 0.015	1.077 ± 0.005*
L2-L4	0.988 ± 0.006	0.965 ± 0.010	0.981 ± 0.026	0.967 ± 0.009*
Femoral neck	0.758 ± 0.004	0.739 ± 0.006*	0.736 ± 0.017	0.739 ± 0.006†
Trochanter	0.673 ± 0.004	0.655 ± 0.006*	0.659 ± 0.017	0.655 ± 0.006*
Ward's triangle	0.559 ± 0.004	0.534 ± 0.007*	0.532 ± 0.020	0.534 ± 0.007‡
Premenopausal women				
No. (%)	200 (71.7)	70 (25.1)	9 (3.2)	79 (28.3)
Age (yr)	46.2 ± 0.3	45.6 ± 0.5	49.9 ± 1.5§	46.1 ± 0.5
BMI (kg/m²)	22.8 ± 0.2	22.8 ± 0.4	22.7 ± 1.1	22.8 ± 0.4
Fracture (%)	23 (11.5)	8 (11.4)	3 (33.3)	11 (13.9)
BMD values (g/cm²)				
Total body	1.091 ± 0.006	1.102 ± 0.010	1.088 ± 0.028	1.100 ± 0.009
L2-L4	1.019 ± 0.009	1.035 ± 0.014	1.031 ± 0.041	1.035 ± 0.014
Femoral neck	0.770 ± 0.007	0.775 ± 0.016	0.793 ± 0.033	0.777 ± 0.011
Trochanter	0.654 ± 0.006	0.664 ± 0.011	0.689 ± 0.030	0.667 ± 0.010
Ward's triangle	0.659 ± 0.009	0.654 ± 0.015	0.693 ± 0.042	0.658 ± 0.014
Postmenopausal women				
No. (%)	563 (69.7)	214 (26.5)	31 (3.8)	245 (30.3)
Age (yr)	63.9 ± 0.4	64.1 ± 0.6	65.0 ± 1.5	64.2 ± 0.5
BMI (kg/m²)	23.0 ± 0.1	22.8 ± 0.2	23.3 ± 0.6	22.9 ± 0.2
Fracture (%)	114 (20.2)	45 (21.0)	8 (25.8)	53 (21.6)
BMD values (g/cm²)				
Total body	0.920 ± 0.004	0.915 ± 0.006	0.914 ± 0.016	0.915 ± 0.006
L2-L4	0.808 ± 0.006	0.806 ± 0.009	0.841 ± 0.025	0.810 ± 0.009
Femoral neck	0.645 ± 0.004	0.642 ± 0.006	0.637 ± 0.016	0.641 ± 0.006
Trochanter	0.540 ± 0.004	0.538 ± 0.006	0.533 ± 0.016	0.537 ± 0.006
Ward's triangle	0.452 ± 0.005	0.451 ± 0.008	0.461 ± 0.022	0.452 ± 0.008

NOTE. Data are means ± SE. BMD values are adjusted for age.
*$P < .05$, †$P < .01$, ‡$P < .005$ *v CC*.
§$P < .05$ *v CC* or *CT*.

adjustment for age by the least squares method in a general linear model. A P value $< .05$ was considered statistically significant.

RESULTS

Age, body mass index (BMI), and the prevalence of nontraumatic fractures did not differ among −1562C→T genotypes in men or in premenopausal or postmenopausal women (Table 1). We compared BMD values among the 3 genotypes (*CC*, *CT*, and *TT*), as well as between 2 groups of genotypes in dominant (*CC* and *CT* + *TT*) and recessive (*CC* + *CT* and *TT*) genetic models to examine the effect of the *T* allele on BMD. BMD for the total body, lumbar spine, femoral neck, trochanter, or Ward's triangle was significantly lower in the combined group of men with the *CT* or *TT* genotypes or in men with the *CT* genotype than in those with the *CC* genotype (Table 1). The differences in BMD between men with the *CC* genotype and those with either the *CT* or *TT* genotypes (expressed as a percentage of the corresponding larger value) were 1.5% for the total body, 2.2% for the lumbar spine, 2.8% for the femoral neck, 2.7% for the trochanter, and 5.2% for Ward's triangle. For premenopausal or postmenopausal women, BMD did not differ among −1562C→T genotypes (Table 1).

DISCUSSION

We previously showed that the −1607G→GG polymorphism of *MMP1* was associated with BMD at the radius in postmenopausal women,[6] with the *GG* genotype, which exhibits an increased transcriptional activity,[7] representing a risk factor for reduced BMD. The T allele of the −1562C→T polymorphism in the promoter of *MMP9* also exhibits higher transcriptional activity than does the *C* allele.[5] A 9-bp sequence (−1567 to −1559) containing the −1562C→T site has been suggested to function as an important regulatory element by serving as a binding site for a transcriptional repressor protein. In addition, the serum concentration of MMP-9 was shown to

be higher in individuals with the *TT* genotype than in those with the *CC* or *CT* genotypes.[5] We have now shown that the −1562C→T polymorphism of *MMP9* was associated with BMD at various sites in Japanese men, with the *T* allele being related to reduced bone mass. Given that MMP-9 degrades collagen in the bone matrix, an increased activity of this protease might be expected to result in reduced bone mass. Our results are thus consistent with the previous observations that the *T* allele of *MMP9* exhibits higher transcriptional activity and is associated with a higher serum concentration of the encoded protein.[5]

Given that BMD values for the total body, lumbar spine, femoral neck, trochanter, and Ward's triangle in men with the *TT* genotype were similar to those in men with the *CT* genotype, the *T* allele may exert a dominant effect on BMD. The lack of statistical significance for differences in BMD between the *CC* and *TT* genotypes may be attributable to the small number of subjects with the *TT* genotype (n = 40), compared with the number of those with the *CT* genotype (n = 280). This polymorphism was associated with BMD in men but not in women. The reason for this gender difference remains unclear, but differences in the concentrations of estrogen and other sex hormones between men and women might be contributing factors. Although it is possible that the −1562C→T polymorphism of *MMP9* is in linkage disequilibrium with polymorphisms of other nearby genes that are actually responsible for reduced BMD, our present results suggest that this polymorphism of *MMP9* is associated with BMD in Japanese men.

REFERENCES

1. Wucherpfennig AL, Li YP, Stetler-Stevenson WG, et al: Expression of 92 kD type IV collagenase/gelatinase B in human osteoclasts. J Bone Miner Res 9:549-556, 1994

2. Okada Y, Naka K, Kawamura K, et al: Localization of matrix metalloproteinase 9 (92-kilodalton gelatinase/type IV collagenase = gelatinase B) in osteoclasts: Implications for bone resorption. Lab Invest 72:311-322, 1995

3. Engsig MT, Chen Q-J, Vu TH, et al: Matrix metalloproteinase 9 and vascular endothelial growth factor are essential for osteoclast recruitment into developing long bones. J Cell Biol 151:879-889, 2000

4. McClelland P, Onyia JE, Miles RR, et al: Intermittent administration of parathyroid hormone (1-34) stimulates matrix metalloproteinase-9 (MMP-9) expression in rat long bone. J Cell Biochem 70: 391-401, 1998

5. Zhang B, Ye S, Herrmann S-M, et al: Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis. Circulation 99:1788-1794, 1999

6. Yamada Y, Ando F, Niino N, Shimokata H: Association of a polymorphism of the matrix metalloproteinase-1 gene with bone mineral density. Matrix Biol 21:389-392, 2002

7. Rutter JL, Mitchell TI, Buttice G, et al: A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. Cancer Res 58:5321-5325, 1998

Mitochondrial ALDH2 Deficiency as an Oxidative Stress

SHIGEO OHTA,[a] IKUROH OHSAWA,[a] KOUZIN KAMINO,[a,c] FUJIKO ANDO,[b] AND HIROSHI SHIMOKATA[b]

[a]*Department of Biochemistry and Cell Biology, Institute of Development and Aging Sciences, Graduate School of Medicine, Nippon Medical School, Kosugi, Kawasaki, Kanagawa, 211-8533 Japan*

[b]*Department of Epidemiology, National Institute for Longevity Sciences, Obu, Aichi, 474-8522 Japan*

ABSTRACT: **Mitochondrial aldehyde dehydrogenase 2 (ALDH2) plays a major role in ethanol metabolism. It is involved in acetaldehyde detoxification. A polymorphism of the ALDH2 gene is specific to North-East Asians. Sensitivity to ethanol is highly associated with this polymorphism (*ALDH2*2* allele), which is responsible for a deficiency of ALDH2 activity. We first show that this deficiency influences the risk for late-onset Alzheimer's disease (LOAD) by a case-control study in a Japanese population. In a comparison of 447 patients with sex, age, and region-matched non-demented controls, the genotype frequency for the *ALDH2*2* allele was significantly higher in the patients than in the controls (P=0.001). Next, we examined the combined effect of the *ALDH2*2* and the apolipoprotein E4 allele (*APOE-ε4*), which has been confirmed to be a risk factor for LOAD. The *ALDH2*2* allele more significantly affected frequency and age at onset in patients with *APOE-ε4* than in those without it. These results indicate that the ALDH2 deficiency is a risk factor for LOAD, acting synergistically with the *APOE-ε* allele. Next, to elucidate the molecular mechanism involved, we obtained *ALDH2*-deficient cell lines by introducing mouse mutant ALDH2 cDNA into PC12 cells. We speculate that ALDH2 may act to oxidize toxic aldehyde derivatives. Then, we found that the *ALDH2*-deficient transfectants were highly vulnerable to exogenous 4-hydroxy-2-nonenal, an aldehyde derivative generated from peroxidized fatty acids. In addition, the *ALDH2*-deficient transfectants were sensitive to oxidative insult induced by antimycin A, accompanied by an accumulation of proteins modified with 4-hydroxy-2-nonenal. Mitochondrial ALDH2 functions as a protector against oxidative stress.**

KEYWORDS: **aldehyde dehydrogenase; ethanol metabolism; Alzheimer's disease; oxidative stress; 4-hydroxy-nonenal; peroxide**

Address for correspondence: Shigeo Ohta, Department of Biochemistry and Cell Biology, Institute of Development and Aging Sciences, Graduate School of Medicine, Nippon Medical School, Kosugi, Kawasaki, Kanagawa, 211-8533 Japan. Voice: +81-44-733-9267; fax: +81-44-733-9268.

ohta@nms.ac.jp

[c]Present address: Division of Psychiatry and Behavioral Proteomics, Department of Post-Genomics and Diseases, Osaka University Graduate School of Medicine, Suita, Osaka, 565-0871 Japan.

Ann. N.Y. Acad. Sci. 1011: 36–44 (2004).
doi: 10.1196/annals.1293.004

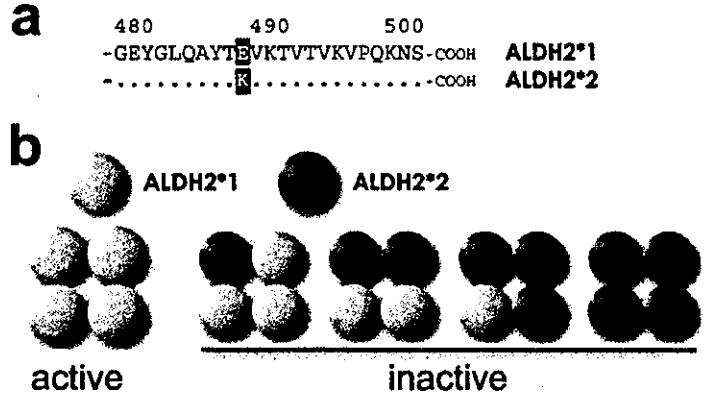

FIGURE 1. A polymorphism specific to North-East Asians in the ALDH2 gene. (a) C-terminal amino acid sequences of the active and inactive subunits termed *ALDH2*1* and *ALDH2*2*. (b) Schematic representation of a homotetrameric enzyme, ALDH2. All tetramers containing at least one *ALDH2*2* subunit are inactive.

INTRODUCTION

A Polymorphism of Aldehyde Dehydrogenase 2 Specific to North-East Asians

Mitochondrial aldehyde dehydrogenase 2 (ALDH2) is located in the matrix of mitochondria and plays a major role in metabolizing acetaldehyde produced from ethanol into acetate. A mutant allele, *ALDH2*2*, has a single point mutation (G/A) in exon 12 of the active *ALDH2* gene and is confined to North-East Asians. The mutation results in the substitution of glutamic acid 487 with lysine (E487K), acting in a dominant negative fashion (FIG. 1). Individuals with the *ALDH2*2* allele exhibit the alcohol flushing syndrome, attributable to an elevated blood acetaldehyde level. The *ALDH2*2* allele has been also reported to affect the metabolism of other aldehydes such as benzaldehyde, which is a metabolite of toluene, and chloroacetaldehyde, which is generated during the metabolism of vinyl chloride. However, the risks have been mainly associated with alcohol consumption. We directed our attention to the genetic role of ALDH deficiency to help us understand the physiological role of ALDH2.

ASSOCIATION OF ALZHEIMER'S DISEASE WITH ALDH2 DEFICIENCY

A Large-Scale Case-Control Study on Alzheimer's Disease with ALDH2 Deficiency

Late-onset Alzheimer's disease (LOAD) is a complex disease caused by multiple genetic and environmental factors upon aging. It was pointed out that alcohol intake

could affect the development of LOAD, because ethanol and its metabolite, acetaldehyde, are directly neurotoxic, and patients with a history of alcohol abuse show alterations in neurotransmitting molecules in the brain, such as the muscarinic cholinergic receptor and serotonin. On the other hand, epidemiological studies have provided conflicting results, which may be explained by genetic factors that modify ethanol metabolism and potentially influence alcohol-drinking behavior.

To understand the genetic effect of *ALDH2**2, we performed a large-scale case-control study in patients with LOAD by examining the frequency of *ALDH2**2. Patients with LOAD and controls in three areas of Japan (447 patients and as many controls) were examined to find the effect of the *ALDH2**2 allele on the risk for LOAD.[1] The controls were strictly selected to match the patients in age, gender, and area. Since the ALDH2 deficiency appears in a dominant-negative fashion, homozygous and heterozygous carriers of the allele were combined in evaluating the risk for LOAD. The frequency of carriers with the *ALDH2**2 allele (*1/2* and *2/2*) was significantly higher in the patients than in the controls [odds ratio (O.R.)=1.6, P=0.001]. This trend was evident in both males (O.R.=1.9, P=0.01) and females (O.R.=1.4, P=0.02).

Synergistic Effect by APOE-ε4

To confirm the effect of *ALDH2**2 on LOAD, we examined the interaction between the *APOE-ε4* and *ALDH2**2 alleles.[1] Since *APOE-ε4* has been established as a risk of LOAD, a synergistic effect of the two genes would strongly support that the *ALDH2**2 allele is also a risk factor. Harboring of the *ALDH2**2 allele synergistical-

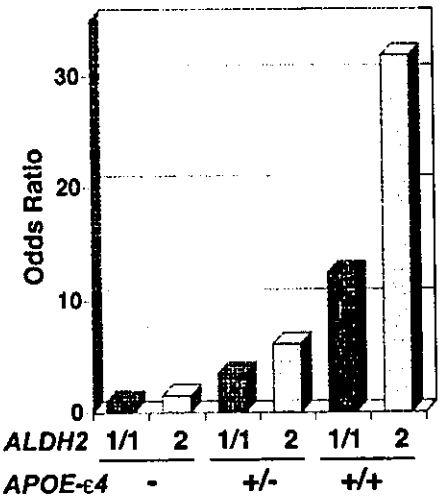

FIGURE 2. Synergistic effect on onset of LOAD by *ALDH2**2 with *APOE-ε4*. Relative risks of LOAD were estimated by the frequencies of patients (N=447) and controls (N=447) with each allele of the *APOE4* and *ALDH2* gene. *ALDH2* 1/1, carrier of homozygous *ALDH2**1; *ALDH2**2, carrier of homozygous and heterozygous *ALDH2**2; *APOE* ε4 −, no *APOE* ε4; +/−, heterozygous *APOE* ε4; and +/+, homozygous *APOE* ε4.

ly increased the odd ratios of patients with the *APOE-ε4* allele (FIG. 2), supporting that the *ALDH2*2* allele is indeed a risk factor for LOAD.

Next, the effect of these alleles on age at onset was examined. In all patients with LOAD, the difference in age at onset was independent of the *APOE-ε4* allele. In contrast, those patients with *ALDH2*2* (*1/2* or *2/2*) and homozygous for *APOE-ε4* showed a significantly earlier onset than other patients. In addition, a dosage effect of the *ALDH2*2* allele on age at onset showed a significant trend in patients homozygous for the *APOE-ε4* allele by regression analysis (P=0.028).

Since logistic regression analysis indicates a significant effect of the *ALDH2*2* allele (P=0.002), the allele is an independent risk factor for LOAD from the *APOE-ε4* allele. Therefore, we conclude that the *ALDH2*2* allele is an independent risk for LOAD and shows synergistic effects with *APOE-ε4* in affecting not only the frequency of LOAD, but also the age at onset of Alzheimer's disease.

PHENOTYPE OF INDIVIDUALS WITH THE ALDH2 DEFICIENCY

Geriatric diseases, including LOAD, are associated with many factors; genetic, life-style–related, physiological, medical, nutritional, and psychological. Thus, it is important to clarify the contributions of genetic factors and other basic background factors. In 1997, we started gene-related investigations into various geriatric diseases in the National Institute for Longevity Sciences, Longitudinal Study of Aging (NILS-LSA).[2] The subjects numbered 2,259. They were community-dwelling males and females aged 40–79 years randomly selected from the area around NILS.

We examined the association of the *ALDH2*-deficient genotype with various other factors evaluated in NILS-LSA.[3] In addition to biochemical analyses of blood and urine, renal and liver functions, serum proteins and lipids, and a complete blood count, lipid peroxide (LPO), and geriatric disease markers were also examined. Several serum proteins, lipids, and LPO levels showed differences between the non-defective (*ALDH2*1/1*) and defective (*ALDH2*1/2* and *ALDH2*2/2*) ALDH2 individuals. However, these biochemical evaluations are notoriously affected by alcohol-drinking behavior. Indeed, subjects with the *ALDH2*1/1* genotype drank alcohol more frequently than those with *ALDH2*1/2* and *2/2*. Thus, we excluded the effects of alcohol-drinking behavior from the association of the *ALDH2*-deficient genotype with the evaluation. Data were analyzed with an adjustment for alcohol consumption by the least squares method in a general linear model. We found that the concentration of LPO in females differed significantly according to *ALDH2* genotype. The concentration was higher in females carrying at least one *ALDH2*2* allele (2.922 nmol/mL) than in those carrying *ALDH2*1/1* (2.781 nmol/mL; P=0.003), raising the possibility that oxidative stress increases in *ALDH2*-deficient individuals.[3]

ALDH2 AS A PROTECTOR AGAINST OXIDATIVE STRESS

Model for Explaining the Role of the ALDH2 Deficiency

Oxidative stress and lipid peroxidation caused by reactive oxygen species (ROS) are reported to play an important role in the pathogenesis of neurodegenerative dis-