ある．中高年者の体重当たりの脚筋力（伸展パワー）は加齢とともに低下する．特に女性の40代の脚筋力は男性の70代とほぼ同等であり，筋力を保持することは女性において，より重要だと考えられる．しかし，運動習慣の割合は20代から40代の女性で20%前後と低い（平成12年度国民栄養調査）．仕事，育児，家事などで余暇時間が少ないことが影響していると考えられるが，今後女性の要介護期間を減らす意味でも，女性の運動習慣が増えるような社会的支援が必要である．

また，藤原らの研究によれば[8]，一般地域住民においてIADLの低下に先駆けて，知的能動性や社会活動が低下する．知的関心の低下や社会参加が減ることは社会適応を低下させ，閉じこもりの原因となると考えられる．高齢者の社会参加を増やし，高齢者の生き甲斐を創世するような社会基盤の整備が，将来の要介護人口を減らし，より健康的な高齢社会を形成するために必要である．

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Abstract

**Strategies to reduce bed-ridden or house-bound elderly people in Japan**

Fujiko Ando

Preventive medicine is supposed to be important for reducing bed-ridden ('netakiri', in Japanese) or frail elderly people. Previous studies showed that only about 30% of the bed-ridden elderly had decreased their ADL levels directly due to diseases, such as cerebrovascular disease or hip fracture. One of the other important causes of 'Netakiri' is disused syndrome. A few weeks after staying in bed, not only muscle power but also bone mineral density and intellectual interest often decrease in the elderly. Rehabilitation in daily life is expected to prevent disused syndrome. House-bound ('tojikomori', in Japanese) is supposed to be another cause of reduction of ADL. There are miscellaneous causes of tojikomori. Aging is one of the most important factors, but cannot be modified. Physical, mental, social or environmental factors are also important. Participation in social activity, improvement of intellectual interest and habitual physical excise, as well as prevention of diseases, is expected to be useful for preventing 'tojikomori' and 'netakiri' in the elderly.

**Key words**: *House-bound, Bed-ridden, Frail elderly, Preventive medicine*
(Jpn J Geriat 2004；41：61—64)

Department of Epidemiology National Institute for Longevity Sciences

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′-AAACCAGCCTGGT-CAACGTA-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

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*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.*

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*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.

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# Mitochondrial ALDH2 Deficiency as an Oxidative Stress

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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**

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

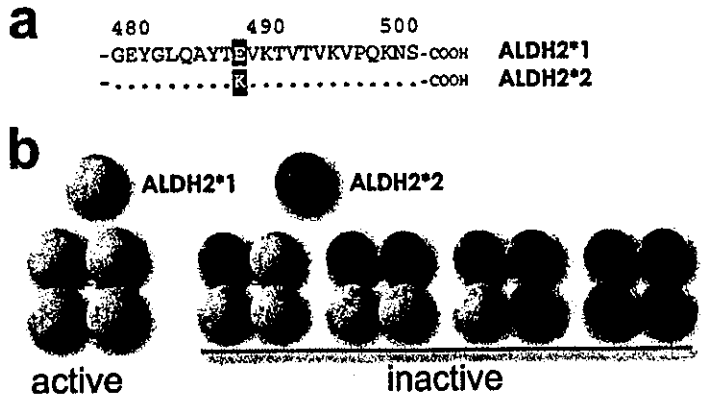


**FIGURE 1.** A polymorphism specific to North-East Asians in the ALDH2 gene. (a) C-terminal amino acid sequences of the active and inactive subunits termed *ALDH2*1* and *ALDH2*2*. (b) Schematic representation of a homotetrameric enzyme, ALDH2. All tetramers containing at least one *ALDH2*2* subunit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

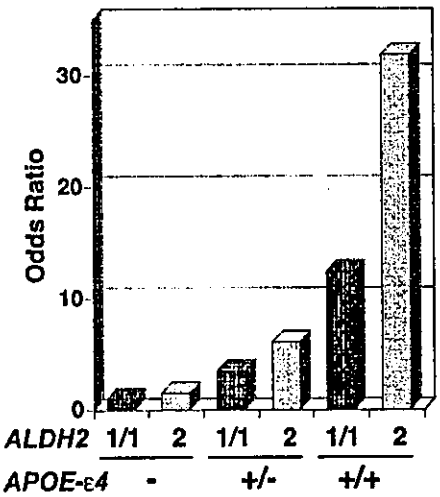


**FIGURE 2.** Synergistic effect on onset of LOAD by *ALDH2**2 with *APOE-ε4*. Relative risks of LOAD were estimated by the frequencies of patients ($N$=447) and controls ($N$=447) with each allele of the *APOE4* and *ALDH2* gene. *ALDH2* 1/1, carrier of homozygous *ALDH2**1*; *ALDH2**2, carrier of homozygous 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-

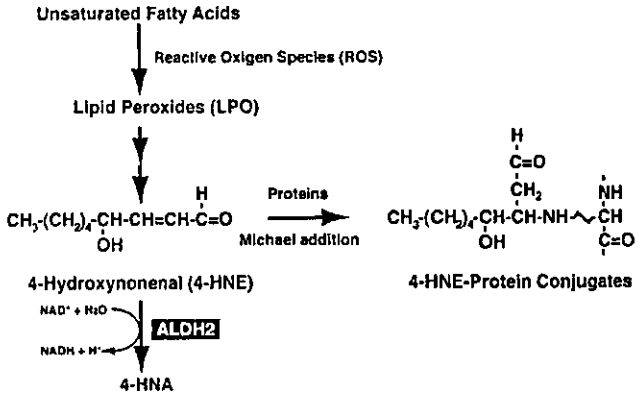


**FIGURE 3.** Involvement of ALDH2 in the metabolism of 4-HNE.

eases, including Alzheimer's disease. A major source of ROS is the mitochondrially derived superoxide anion radical, which induces membrane lipid peroxidation, thereby generating reactive aldehydes, including malondialdehyde (MALD) and trans-4-hydroxy-2-nonenal (4-HNE). A strong electrophile, 4-HNE, has the ability to readily adduct cellular proteins and may damage the proteins by interacting with lysine, histidine, serine, and cysteine residues.

Thus, we hypothesized that ALDH2 is involved in antioxidant defense through the oxidation of toxic aldehyde derivatives and its deficiency enhances oxidative stress (FIG. 3).

### *Construction of ALDH2-Deficient Cell Lines*

To verify this hypothesis, we obtained ALDH2-deficient PC12 cells by transfection with a dominant-negative form of the mouse *Aldh2* gene.[4] Then, we examined the toxic effect of 4-HNE and found that exposure to 4-HNE resulted in more rapid decrease of viable cells in the ALDH2-deficient population than in control cells (FIG. 4). Exposure to 10 μM 4-HNE for about 2 h resulted in the appearance of round cells. At that time, the percentage of living ALDH2-deficient cells (K6 and K11) was 37% and 35%, whereas that of control cells (PC12, V, and E) was 99%, 85%, and 102%, respectively. Time-course study revealed that one day after exposure to 10 μM 4-HNE, the survival of ALDH2-deficient cells decreased rapidly, whereas that of control cells decreased gradually. The sensitivity of ALDH2-deficient cells to 4-HNE was dose dependent. These findings clearly show that ALDH2-deficient cells are less resistant to exogenous 4-HNE.

### *Effect of Generation of Superoxide on Cytotoxicity*

Next, we tried to generate superoxide anion through exposure to an external insult. Partial inhibition of the mitochondrial electron transport at complex III by low concentrations of antimycin A induces the production of ROS and cell death. To in-

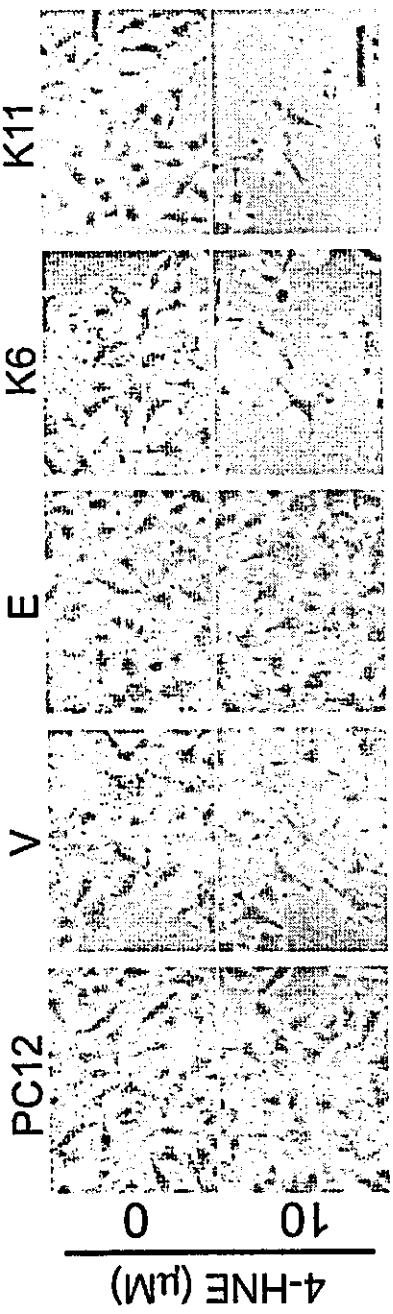


**FIGURE 4.** Rapid cell death of ALDH2-deficient PC12 transfectants after treatment with 4-HNE. PC12 or each transfectant (V, E, K6, or K11) was treated with 10 μM 4-HNE or ethanol (1/1,000 volume of medium) as a control (0 μM). One day after treatment, cells were observed under a phase-contrast microscope (×200). Bar=50 μm.

vestigate the effect of ALDH2 deficiency on cell vulnerability induced by oxidative stress, we examined the cellular toxicity of antimycin A in the ALDH2-deficient and parental cells of PC12.[4] In this experiment, we confirmed that the generation of ROS did not depend upon the type of transfectant. Then, we examined whether the accumulation of 4-HNE induced by the ROS differed between the ALDH2-deficient and normal cells. The accumulation after the exposure to antimycin A was measured with an anti–4-HNE antibody in immunocytochemical assays. A day after treatment with antimycin A (3 or 10 μg/mL), cellular 4-HNE immunoreactivity increased only in ALDH2-deficient cells, K6 and K11, but not in control cells (FIG. 5). These results strongly suggest that the ALDH2 deficiency caused the intracellular accumulation of 4-HNE, resulting in cell death.

ALDH2 deficiency was found to contribute to risks of diabetes,[5] cancer,[6] hypertension,[7,8] and myocardial infarction.[9] However, the risks have been mainly attributed to the association with alcohol consumption and the increase in the acetaldehyde concentration. In contrast, this study proposes that ALDH2 can contribute to the pathogenesis of various geriatric diseases by an alternative pathway, that is, the detoxification of cytotoxic products of lipid peroxidation.

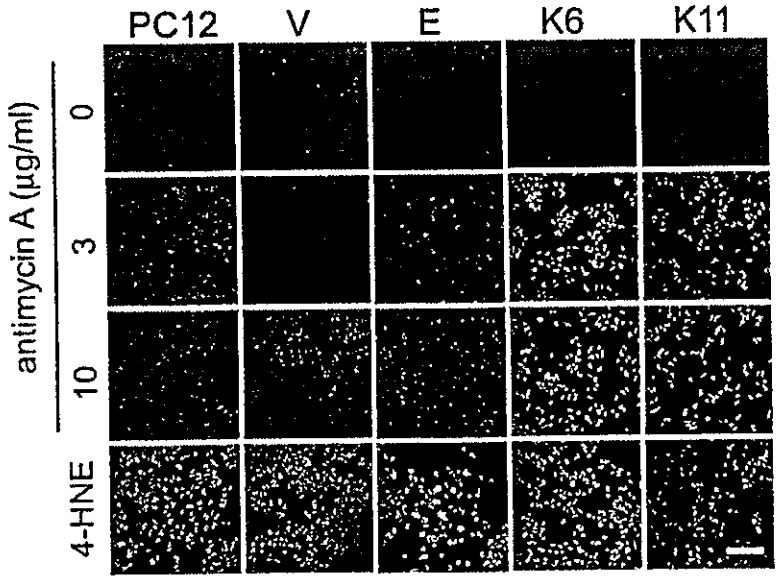


**FIGURE 5.** Accumulation of 4-HNE by superoxide. Cells were treated with the indicated concentration of antimycin A or 1 μM 4-HNE, and incubated for 24 h. After fixation, cells were stained with anti-4-HNE antibody. Bar=200 μm.

## DISCUSSION OF THE ROLE OF ALDH2 DEFICIENCY IN OXIDATIVE STRESS

It has been shown that patients with Alzheimer's disease homozygous for *APOE-ε4* have greater 4-HNE adduct immunoreactivity associated with neurofibrillary tangles than those with other APOE genotypes. Studies of the interactions of *APOE* proteins with 4-HNE showed that the isoforms differ in the amount of 4-HNE they can bind, with the order ε2>ε3>ε4.[10] This correlated with the different abilities of APOE isoforms to protect against apoptosis induced by 4-HNE in cultured neurons. Our case-control study has revealed that ALDH2 deficiency is a risk factor for LOAD in a Japanese population, synergistically acting with *APOE-ε4*.[1] When compared with carriers of the *APOE-ε3/ε3* genotype, the risk for LOAD in Japanese subjects with the *APOE-ε4* allele is twice that in Caucasian subjects. The increased risk can partly be explained by the effect of the *ALDH2**2 allele, since this allele is very rare in non-Asian populations. Therefore, we suggest the possibility that in LOAD an enhancement of 4-HNE accumulation in Alzheimer's disease brain caused by ALDH2 deficiency may act synergistically with a weaker activity of APOE-ε4 to protect against neuronal cell death induced by 4-HNE. However, as Japanese patients with Alzheimer's disease are less numerous than Caucasian patients, other risks must overcome that posed by ALDH2 deficiency.

Taken together, our results suggest that mitochondrial ALDH2 functions to protect against oxidative stress. Thus, the metabolism of aldehyde including ALDH2 could be a preventive and therapeutic target in Alzheimer's disease and other neurodegenerative disorders.

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研究報告●16

# 地域在住高齢者の転倒恐怖感に関連する要因の検討

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下方　浩史*

## 1. 背景および目的

高齢者の転倒は，骨折などの身体的外傷だけではなく心理面にも多大な影響を及ぼす。特に転倒に対する心理的反応である転倒恐怖感は，その後の社会活動や余暇活動を制限し，生活の質を低下させる大きな要因になると指摘されている[1,2]。

転倒恐怖感と関連する要因としては，直接の転倒経験よりもむしろ，歩行機能やバランスの障害，骨折経験などの身体状況が有意であることが報告されてきた[2-4]。一方，心理的ケアの重要性[3]や社会活動低下との関連[2,5]も示唆されており，さらに心理・社会的側面を考慮して検討する必要があると考えられる。

本研究では，転倒経験，骨折経験，生活機能などの身体的要因に加えて心理・社会的要因を取り上げ，転倒恐怖感に関連する要因について検討する。

## 2. 方法

### 1．対象

対象は，国立長寿医療研究センター（現・国立長寿医療センター研究所）疫学研究部が行っている「老化に関する長期縦断疫学調査(National Institute for Longevity Sciences-Longitudinal Study of Aging(NILS-LSA))」の第一次調査(1997～2000年)に参加した地域在住高齢者である。NILS-LSAは，年齢および性で層化無作為抽出された地域住民を対象とした，老化と老年病に関する縦断的コホート調査であり，国立療養所中部病院(現・国立長寿医療センター)倫理委員会の了承の下に「調査への参加の文書による同意(informed consent)」の得られた者を対象として行われている[6]。

本研究では，転倒がQOLを脅かす重大な要因になると指摘されている60～79歳の高齢者1,133名の中で，下記の設問すべてに回答しており，認知障害を有する可能性が低い(MMSE≧24)1,025名(男性504名：68.5±5.3歳，女性521名：68.6±5.6歳)を対象とした。

### 2．変数

質問紙法により以下の変数を収集して，コーディングを行った。

結果変数：転倒恐怖感[有(とても怖い・少し怖い)＝1，無(怖くない)＝0)]

説明変数：年代(70歳代＝1，60歳代＝0)，過去1年間の転倒経験(有＝1，無＝0)，骨折経験(有＝1，無＝0)，生活機能[老研式活動能力指標[7]：低(≦10)＝1，高(11≦)＝0]，抑うつ[老人用うつ尺度(GDS)[8]：高(6≦)＝1，低(≦5)＝0]，主観的健康感[不良(非常に悪い・悪い)＝1，良好(非常に良い・良い・普通)＝0]，同居家族(無＝1，有＝0)，仕事(無＝1，有＝0)，趣味(無＝1，有＝0)

### 3．統計解析

$\chi^2$検定によって結果変数と各説明変数との関連性を検討し，有意な関連($p<0.05$)を示した変数を説明変数とするロジスティック回帰分析を行った。なお，これまで

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表1 転倒恐怖感の分布 人数(%)

| | 60歳代 | 70歳代 | 合計 |
|---|---|---|---|
| <男性> | | | |
| 転倒恐怖感有 | 92( 35.4) | 135( 55.3) | 227( 45.0) |
| 転倒恐怖感無 | 168( 64.6) | 109( 44.7) | 277( 55.0) |
| 合計 | 260(100.0) | 244(100.0) | 504(100.0) |
| <女性> | | | |
| 転倒恐怖感有 | 183( 68.5) | 203( 79.9) | 386( 74.1) |
| 転倒恐怖感無 | 84( 31.5) | 51( 20.1) | 135( 25.9) |
| 合計 | 267(100.0) | 254(100.0) | 521(100.0) |

に転倒恐怖感の分布や関連要因に性差が確認されている[3]ことから，性別に解析した。統計解析にはSAS release 8.2を用いた。

## 3．結果

### 1．転倒恐怖感の分布(表1)

転倒恐怖感を有する高齢者は，男性で45.0%，女性では74.1%であり，男性よりも女性の方がその割合が高かった($\chi^2(1)=89.9$, $p<0.001$)。また，男女ともに，60歳代よりも70歳代の方が転倒恐怖感を有する割合が高かった(男性$\chi^2(1)=20.2$, $p<0.001$，女性$\chi^2(1)=8.8$, $p<0.001$)。

### 2．転倒恐怖感の関連要因(表2)

男性において，$\chi^2$検定によって転倒恐怖感と有意な関連を示した変数は，年代・転倒経験・抑うつ・主観的健康感・仕事であった。これらを説明変数としたロジスティック回帰分析を行った結果，年代(70歳代)・転倒経験(有)・仕事(無)，抑うつ(高)の場合に転倒恐怖感を有する傾向が高かった。一方，女性において，$\chi^2$検定によって転倒恐怖感と有意な関連を示した変数は，年代・転倒経験・骨折経験・生活機能・抑うつ・主観的健康感・趣味であった。これらを説明変数としたロジスティック回帰分析を行った結果，骨折経験(有)，年代(70歳代)・主観的健康感(不良)，抑うつ(高)の場合に転倒恐怖感を有する傾向が高かった。

表2 ロジスティック回帰分析結果
結果変数：転倒恐怖感(無=0，有=1)

| | Odds ratio | 95%CI |
|---|---|---|
| <男性> | | |
| 年代(70歳代) | 1.77** | 1.22～2.59 |
| 転倒経験(有) | 2.08** | 1.21～3.55 |
| 抑うつ(高) | 1.90* | 1.14～3.16 |
| 主観的健康感(不良) | 1.34 | 0.77～2.32 |
| 仕事(無) | 1.94*** | 1.31～2.87 |
| <女性> | | |
| 年代(70歳代) | 1.72** | 1.14～2.62 |
| 転倒経験(有) | 1.51 | 0.88～2.56 |
| 骨折経験(有) | 2.25** | 1.29～3.94 |
| 生活機能(低) | 1.3 | 0.54～3.10 |
| 抑うつ(高) | 1.88† | 0.99～3.58 |
| 主観的健康感(不良) | 2.23* | 1.04～4.74 |
| 趣味(無) | 1.25 | 0.78～1.99 |

***：$p<0.001$，**：$p<0.01$，*：$p<0.05$，†：$p<0.10$

注) $\chi^2$検定によって転倒恐怖感と有意な関連($p<0.05$)を示した項目を説明変数として分析を行った。

## 4．考察

転倒恐怖感を有する対象者は全体で59.8%，男性で45.0%，女性で74.1%であり，地域高齢者を対象としたHowlandら[4]，鈴木ら[3]の報告と類似する傾向が確認された。

今回の結果は横断的調査から得られたものであり，転倒恐怖感と諸変数間の因果関係は特定できないが，男性・女性ともに抑うつとの有意な関連がみられたことから，転倒恐怖感への対処を検討する際には，転倒に対する心理的反応だけではなく，全般的な心理状態を考慮に入れる必要があると思われる。また，男性において仕事との関連が示されたことは，退職期に当たる60歳以降の社会参加が転倒恐怖感を軽減する可能性を示唆している。この社会的側面については，男性・女性ともに多くの高齢者が社会参加や余暇活動への意欲をもっている現状[9]を考えると，仕事以外の社会活動との関連からも検討する必要がある。さらに，男性・女性特有の要因が存在することが示されたことから，転倒恐怖感を軽減するケアを進める際には，性別を考慮する重要性が示唆される。

今後，さらに縦断的調査を行い，転倒恐怖感に伴うQOL指標の変化や，変数間の因果関係について検討する必要がある。

## 5．結語

地域在住高齢者の転倒恐怖感は心理・社会的側面と関連すること，男性・女性特有の要因が存在することが示された。

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## Brief Genetic Analysis

# Association of Cholecystokinin 1 Receptor and $\beta_3$-Adrenergic Receptor Polymorphisms with Midlife Weight Gain

*Michiko Koda,*† Fujiko Ando,† Naoakira Niino,† Hiroshi Shimokata,† Kyoko Miyasaka,‡ and Akihiro Funakoshi§*

**Abstract**

KODA, MICHIKO, FUJIKO ANDO, NAOKIRA NIINO, HIROSHI SHIMOKATA, KYOKO MIYASAKA, AND AKIHIRO FUNAKOSHI. Association of cholecystokinin 1 receptor and $\beta_3$-adrenergic receptor polymorphisms with midlife weight gain. *Obes Res.* 2004;8:1212–1216.

We investigated the relationship of polymorphisms in the *cholecystokinin 1 receptor* [*CCK1R*; *G* to *T* (n-128), *A* to *G* (n-81)] and the *$\beta_3$-adrenergic receptor* (*$\beta_3$-AR*; Trp64Arg) with midlife weight gain. The participants were 1012 Japanese men and women (40 to 59 years of age). Their weight at 18 years old was obtained from a questionnaire. Weight change was defined as the current weight minus the weight at 18 years old. Subjects were grouped into four categories by these genotypes: W/W = noncarriers, W/H = *$Arg^{64}$* carriers of the *$\beta_3$-AR*, H/W = *T* (n-128) or *G* (n-81) carriers of the *CCK1R*, H/H = *T* (n-128) or *G* (n-81) and *$Arg^{64}$* carriers. In men, the interaction between the *CCK1R* and *$\beta_3$-AR* polymorphisms was significant (two-way ANOVA, $p < 0.05$), but neither the *CCK1R* nor the *$\beta_3$-AR* was individually associated with weight gain. The H/H group showed a higher possibility of weight gain of 10 kg or more compared with the W/W group in men. The odds ratio for weight gain (≥10 kg) of H/H was 2.54 (95% confidence interval: 1.50 to 4.30) compared with W/W. In women, neither main effect nor interaction was significant. These results suggest that the combination of *CCK1R* and the *$\beta_3$-AR* polymorphisms is a contributing factor for midlife weight gain in men.

**Key words: combination of polymorphism, body weight gain, middle-aged men**

Age-related increases in body weight in young adult men and postmenopausal women have been reported. Weight gain is as harmful to the health as being overweight. In a previous study, weight gain from 20 years of age was closely associated with cardiovascular risk factors in middle-aged men (1), and weight gain from 18 years of age was associated with coronary heart disease risk in women (2). According to a Japanese national cross-sectional survey in 1999 (3), although the rate of excess weight (BMI ≥ 25 kg/m$^2$) was 19.2% in those 20 to 29 years old, it increased to 29.6% in those 50 to 59 years old for men. In women, it was 7.3% in those 20 to 29 years old and 27.5% in those 50 to 59 years old.

There are several causes associated with weight gain, such as smoking, physical activity during leisure, alcohol consumption, and genetic factors (4–6). Regarding obesity, we reported the possibility that the polymorphism of the *cholecystokinin 1 receptor* (*CCK1R*)[1] gene may be related to an increase in body fat content in middle-aged and elderly people (7). Cholecystokinin (CCK) is a peptide hormone found in the central nervous system and gastrointestinal tract. *CCK1R* has been shown to mediate the CCK-induced suppression of food intake (8), and the peripheral administration of *CCK1R* antagonists increased food intake (9). However, Hamann et al. (10) found no evidence for its association with early-onset obesity in children and adolescents.

Received for review June 27, 2003.
Accepted in final form June 11, 2004.
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[1] Nonstandard abbreviations: CCK1R, cholecystokinin 1 receptor; CCK, cholecystokinin; *$\beta_3$*-AR, *$\beta_3$*-adrenergic receptor; NILS-LSA, National Institute for Longevity Sciences-Longitudinal Study of Aging.

The $\beta_3$-*adrenergic receptor* ($\beta_3$-*AR*) genotype has also been cited as a gene candidate related to obesity (6,11,12), and it is involved in the regulation of lipolysis and thermogenesis. Japanese (12), Pima Indians (6), and Alaskan Eskimos (13) have higher frequencies of the $\beta_3$-*AR* gene polymorphism than whites. However, some studies have suggested that the $\beta_3$-*AR* gene is not associated with obesity (13,14). Therefore, we investigated the relationship between *CCK1R* and $\beta_3$-*AR* gene polymorphisms and weight gain from 18 years of age to middle age.

The means and SD of current weight, weight at 18 years, and weight change from 18 years by genotype are shown in Table 1. The means of weight change were 8.2 kg in men and 5.1 kg in women.

Genotype and polymorphism allele frequency distributions for *CCK1R* and $\beta_3$-*AR* are shown by gender in Table 2. These genotype frequencies were found to be in Hardy-Weinberg equilibrium in men and women. Gender differences in those frequency distributions were not significant. The frequency of the *T* (n-128) allele in *CCK1R* was 26% and that of the *G* (n-81) allele was ~40%. Funakoshi et al. (7) has found that there are two sequence changes in human *CCK1R*, a *G* to *T* change in n-128 and an *A* to *G* change in n-81. Six genotypes were identified as wild-type (G/G, A/A), heterozygote type (G/T, A/G), (G/G, A/G), (G/T, G/G), (G/G, G/G), and homozygote type (T/T, G/G). The genotype combinations G/T, A/A; T/T, A/G; and T/T, A/A were not found. On the other hand, the genotype frequency of the $\beta_3$-*AR* gene polymorphism is ~33%, similar to previous studies in other Japanese (12).

Two-way ANOVA was carried out in which weight gain was taken as the dependent variable and the *CCK1R* and $\beta_3$-*AR* polymorphisms were independent variables. Neither *CCK1R* nor $\beta_3$-*AR* was individually associated with weight gain in men. However, the interaction between *CCK1R* and $\beta_3$-*AR* polymorphisms was significant ($p < 0.05$; Table 3). The main effects and the interaction were not significant in women.

Comparisons of the distributions of weight change from 18 years by genotype are shown in Table 4. Of the 564 men, 227 (40%) were noncarriers (W/W), 110 (20%) were $Arg^{64}$ carriers of the $\beta_3$-*AR* (W/H), 149 (26%) were *T* (n-128) or *G* (n-81) carriers of the *CCK1R* (H/W), and 78 (14%) were *T* (n-128) or *G* (n-81) and $Arg^{64}$ carriers (H/H). Of the 548 women, 211 (38%) were W/W, 113 (21%) were W/H, 158 (29%) were H/W, and 66 (12%) were H/H. The frequency of weight gain (≥10 kg) was 40% for men and 24% for women. The distribution of weight change in men was different among the genotypes ($p < 0.01$). The frequency of a weight gain of ≥10 kg was higher in the H/H group than in the other three groups. The distribution in women was not different.

Finally, the risk of weight gain (≥10 kg) was estimated using multiple logistic regression analysis in men (Table 5).

**Table 1.** Characteristics of participants by gender

| | Men ($n$ = 564) | Women ($n$ = 548) |
|---|---|---|
| Height | 164.1 ± 5.9 | 154.1 ± 4.9 |
| Current weight | 65.0 ± 8.7 | 54.1 ± 8.0 |
| Weight at 18 years | 56.8 ± 6.7 | 48.9 ± 6.0 |
| Weight change | 8.2 ± 7.4 | 5.1 ± 7.7 |

Mean ± SD.

The odds ratio of the H/H group was significantly higher [2.54 (95% confidence interval: 1.50 to 4.30)] compared with that of the W/W group. However, in men with W/H or H/W, the odds ratios were not significant.

These results showed that the combination of *CCK1R* and $\beta_3$-*AR* polymorphisms was associated with a weight gain of ≥10 kg from 18 years of age in men. Hamann et al. (10) did not find that the *CCK1R* polymorphism was associated with early-onset obesity in children and adolescents. Although excess energy from increased food intake may be used for growth in a child, it is not usually used for growth in adults. After maturing, the polymorphism of the *CCK1R* gene may have an important role as a regulator of food intake. $\beta_3$-*AR* is involved in the regulation of lipolysis and thermogenesis. The resting metabolic rate in *Arg64* homozygotes is significantly lower than in Trp64 homozygotes (15). Moreover, $\beta_3$-*AR* is expressed in visceral fat in humans (16), and visceral fat increases with advancing age (17). Therefore, in men carrying the *T* or *G* allele of the *CCK1R* and $Arg^{64}$ allele in $\beta_3$-*AR*, food intake may increase, but extra energy may not burn, leading to weight gain.

However, neither *CCK1R* nor $\beta_3$-*AR* was individually associated with weight gain. *CCK1R* or $\beta_3$-*AR* alone was not likely to be a strong independent contributing factor of weight gain. Therefore, the results of the association between a single gene and weight gain in many previous studies have been contradictory. A combination of polymorphisms in two or more candidate genes may contribute to weight gain (e.g., the $\beta_3$-*AR* and uncoupling protein gene) (18,19). The simultaneous existence of two polymorphisms was associated with weight gain.

It remains unclear why these results were revealed only in men. For women, the physiological and environmental factors are relatively strong (e.g., pregnancy, parity, and menopause involve hormonal changes) (20). Furthermore, women may try more frequently to lose weight, and these factors may be stronger than genetic factors.

There are some limitations in this study. First, there may be other factors related to body weight. Smoking influences weight and weight change (4), and we, therefore, performed an analysis excluding smokers. The results were similar to

**Table 2.** Genotype and allele frequencies for *CCK1R* and $\beta_3$-*AR* polymorphisms by gender

| | | Men ($n$ = 564) | | Women ($n$ = 548) | |
|---|---|---|---|---|---|
| | | Count | Percentage | Count | Percentage |
| *CCK1R* (n-128) | Genotype | | | | |
| | G/G | 415 | 73.6 | 403 | 73.5 |
| | G/T | 134 | 23.8 | 133 | 24.3 |
| | T/T | 15 | 2.7 | 12 | 2.2 |
| | Allele | | | | |
| | *G* | 964 | 85.5 | 939 | 85.7 |
| | *T* | 164 | 14.5 | 157 | 14.3 |
| *CCK1R* (n-81) | Genotype | | | | |
| | A/A | 337 | 59.8 | 324 | 59.1 |
| | A/G | 190 | 33.7 | 185 | 33.8 |
| | G/G | 37 | 6.6 | 39 | 7.1 |
| | Allele | | | | |
| | *A* | 864 | 76.6 | 833 | 76.0 |
| | *G* | 264 | 23.4 | 263 | 24.0 |
| $\beta_3$-*AR* | Genotype | | | | |
| | Trp/Trp | 376 | 66.7 | 369 | 67.3 |
| | Trp/Arg | 161 | 28.5 | 158 | 28.8 |
| | Arg/Arg | 27 | 4.8 | 21 | 3.8 |
| | Allele | | | | |
| | *Trp* | 913 | 80.9 | 896 | 81.8 |
| | *Arg* | 215 | 19.1 | 200 | 18.2 |

the original results. Second, the weight estimate at 18 years of age might not be accurate, because this was assessed only by a questionnaire. Third, weight changes, either up or down, were not ascertained for the period between 18 years of age and the time of this study. We need to research this in the future.

## Research Methods and Procedures

### *Subjects*

The subjects were 564 Japanese men and 548 women, 40 to 59 years of age, who participated in the National Institute for Longevity Sciences–Longitudinal Study of Aging

**Table 3.** Relationship between weight gain and the polymorphisms in *CCK1R* and *β3-AR* (two-way ANOVA)

| | Covariable | | Sum of squares | *df* | *F* | *p* |
|---|---|---|---|---|---|---|
| Men | Main effects | *CCK1R* | 173.73 | 1 | 3.18 | 0.075 |
| | | *β3-AR* | 70.45 | 1 | 1.29 | 0.257 |
| | Interactions | *CCK1R* × *β3-AR* | 228.40 | 1 | 4.18 | 0.042 |
| | Model | | 476.62 | 3 | 2.90 | 0.034 |
| Women | Main effects | *CCK1R* | 62.09 | 1 | 1.06 | 0.304 |
| | | *β3-AR* | 2.08 | 1 | 0.04 | 0.851 |
| | Interactions | *CCK1R* × *β3-AR* | 78.5 | 1 | 1.34 | 0.248 |
| | Model | | 141.58 | 3 | 0.80 | 0.492 |

**Table 4.** Comparison of the distributions of body weight change from 18 years by genotype

| | | | <0 kg | | ≥0 to <10 kg | | ≥10 kg | | *p* for genotype frequencies† |
|---|---|---|---|---|---|---|---|---|---|
| | | Total | Number | Percentage | Number | Percentage | Number | Percentage | |
| Men | W/W* | 227 | 29 | 12.8 | 116 | 51.1 | 82 | 36.1 | |
| | W/H* | 110 | 20 | 18.2 | 51 | 46.4 | 39 | 35.4 | |
| | H/W* | 149 | 19 | 12.8 | 73 | 48.9 | 57 | 38.3 | |
| | H/H* | 78 | 6 | 7.7 | 26 | 33.3 | 46 | 59.0 | 0.005 |
| | Total | 564 | 74 | 13.1 | 266 | 47.2 | 224 | 39.7 | |
| Women | W/W* | 211 | 50 | 23.7 | 112 | 53.1 | 49 | 23.2 | |
| | W/H* | 113 | 28 | 24.8 | 58 | 51.3 | 27 | 23.9 | |
| | H/W* | 158 | 40 | 25.3 | 78 | 49.4 | 40 | 25.3 | |
| | H/H* | 66 | 14 | 21.2 | 36 | 54.6 | 16 | 24.2 | 0.985 |
| | Total | 548 | 132 | 24.1 | 284 | 51.8 | 132 | 24.1 | |

* W/W, (*CCK1R*/$\beta_3$-*AR*) = (G/G, A/A)/(Trp/Trp); W/H, (G/G, A/A)/(Trp/Arg) or (Arg/Arg); H/W, (G/T, A/G), (G/G, A/G), (G/T, G/G) or (G/G, G/G)/(Trp/Trp); H/H, (G/T, A/G), (G/G, A/G), (G/T, G/G) or (G/G, G/G)/(Trp/Arg) or (Arg/Arg).
† Cochran-Mantel-Haenszel statistics.

(NILS-LSA) from November 1997 to April 2000. The NILS-LSA is a comprehensive longitudinal study on aging, which started in November 1997. The design of the NILS-LSA has been described elsewhere (21). Informed consent was obtained from all subjects. The study protocol was approved by the Ethical Committee of Chubu National Hospital.

***Measurements***

Body weight of subjects dressed in underwear only was measured with a digital scale. Weight at 18 years of age was collected by questionnaire. Weight change was defined as the current weight minus the weight at 18 years of age.

Venous blood was collected into tubes containing EDTA (disodium salt; 50 mM), and genomic DNA was isolated with an automated genomic DNA isolation system (NA1000; Kurabo, Osaka, Japan).

The polymorphism of the upstream region of the *CCK1R* gene was determined with a mismatch polymerase chain reaction-restriction fragment length polymorphism method (7). Genotyping of the $\beta_3$-*AR* Trp64Arg polymorphism was determined using polymerase chain reaction-restriction fragment length polymorphism analysis (11). These methods have already been described in detail elsewhere (22).

**Table 5.** Odds ratios (ORs) and 95% confidence intervals (95% CIs) for body weight gain (≥10 kg) in men

| | Case number | Referents number | OR | 95% CI |
|---|---|---|---|---|
| W/W* | 82 | 145 | 1.00 | |
| W/H* | 39 | 71 | 0.97 | 0.60–1.56 |
| H/W* | 57 | 92 | 1.10 | 0.72–1.68 |
| H/H* | 46 | 32 | 2.54 | 1.50–4.30 |

* W/W, (*CCK1R*/$\beta_3$-*AR*) = (G/G, A/A)/(Trp/Trp); W/H, (G/G, A/A)/(Trp/Arg) or (Arg/Arg); H/W, (G/T, A/G), (G/G, A/G), (G/T, G/G) or (G/G, G/G)/(Trp/Trp); H/H, (G/T, A/G), (G/G, A/G), (G/T, G/G) or (G/G, G/G)/(Trp/Arg) or (Arg/Arg).
† Cochran-Mantel-Haenszel statistics.

***Data Analysis***

There were two sequence changes in the *CCK1R*, a *G* to *T* transversion at nucleotide −128 (n-128) and an *A* to *G* change in nucleotide −81 (n-81) (GenBank accession no. D85606) (7). The $\beta_3$-*AR* genotype leads to the replacement of tryptophan by arginine at position 64 ($Trp^{64}Arg$). The genotype distributions were tested for Hardy-Weinberg equilibrium with $\chi^2$ statistics. Gender differences in the genotypic distribution were analyzed using $\chi^2$ statistics. Two-way ANOVA was used to evaluate the effect of the genotype and the interaction between that independent variable and weight gain.

Subjects were grouped into four categories by genotype: W/W, W/H, H/W, and H/H. Values for weight change were also grouped into three categories: <0, 0 to 9.9, and ≥10 kg. The distribution of weight change was tested by Coch-