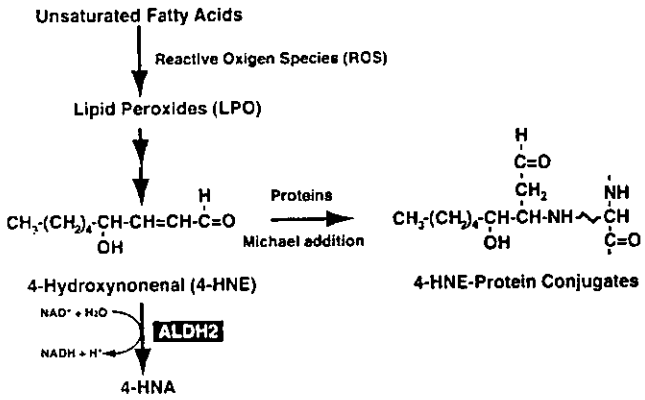

FIGURE 3. Involvement of ALDH2 in the metabolism of 4-HNE.

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.

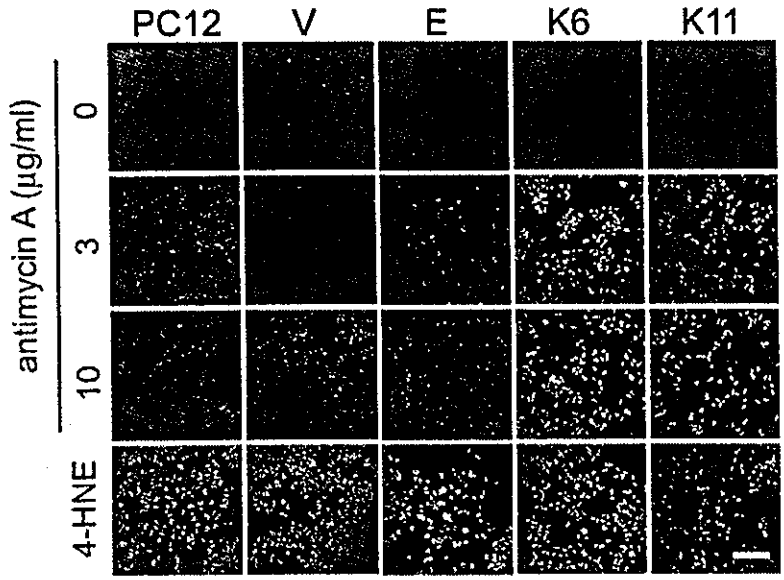

FIGURE 5. Accumulation of 4-HNE by superoxide. Cells were treated with the indicated concentration of antimycin A or 1 μM 4-HNE, and incubated for 24 h. After fixation, cells were stained with anti-4-HNE antibody. Bar=200 μm.

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

χ^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，χ^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，χ^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。

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。

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) χ^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。

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 β_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 β_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 *β_3-adrenergic receptor* (*β_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 *β_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 *β_3-AR* polymorphisms was significant (two-way ANOVA, $p < 0.05$), but neither the *CCK1R* nor the *β_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 *β_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; β_3-AR, β_3-adrenergic receptor; NILS-LSA, National Institute for Longevity Sciences-Longitudinal Study of Aging.

The *β_3-adrenergic receptor* (β_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 β_3-*AR* gene polymorphism than whites. However, some studies have suggested that the β_3-*AR* gene is not associated with obesity (13,14). Therefore, we investigated the relationship between *CCK1R* and β_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 β_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 β_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 β_3-*AR* polymorphisms were independent variables. Neither *CCK1R* nor β_3-*AR* was individually associated with weight gain in men. However, the interaction between *CCK1R* and β_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 β_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 β_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. β_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, β_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 β_3-*AR*, food intake may increase, but extra energy may not burn, leading to weight gain.

However, neither *CCK1R* nor β_3-*AR* was individually associated with weight gain. *CCK1R* or β_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 β_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 β_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
β_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*/β_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 β_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).

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 β_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 χ^2 statistics. Gender differences in the genotypic distribution were analyzed using χ^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-

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*/β_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.

ran-Mantel-Haenszel statistics. The odds ratio for weight gain (≥10 kg) and its 95% confidence interval were estimated using a logistic regression model. The data were analyzed using the SAS statistical software package (release 8.2; SAS Institute, Cary, NC) (23). Probability values below 0.05 were regarded as significant.

Acknowledgments

We thank the participants and colleagues in the National Institute for Longevity Sciences–Longitudinal Study of Aging. This study was supported by a grant-in-aid for Comprehensive Research on Aging and Health from the Ministry of Health, Labor and Welfare Japan.

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Alcohol dehydrogenase 2 variant is associated with cerebral infarction and lacunae

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Abstract—The authors examined the association of the alcohol dehydrogenase 2 (ADH2) genotype with vascular events in community-dwelling Japanese (1,102 men/1,093 women). The allele *ADH2*2* encodes an isozyme with a higher level of activity than ADH2*1. Here, the authors show that the *ADH2*1* carriage is associated with high prevalence of cerebral infarction and lacunae in men. Multiple regression analyses confirmed that the risk of lacunae and cerebral infarction was increased by the *ADH2*1* allele.

NEUROLOGY 2004;63:1711–1713

Alcohol dehydrogenase (ADH) is one of the key enzymes in alcohol metabolism. *ADH2* and *ADH3* have alleles that encode isoenzymes with distinct enzymatic properties.[1] Among Caucasians, a variant *ADH3* allele is found. On the other hand, among Mongoloids, especially the Japanese, about 85% of individuals are carriers of the β2-subunit encoded by the *ADH2*2* allele, compared to only 5% or less of European and white American populations. The β1 (encoded by *ADH2*1*) and β2 subunits (encoded by *ADH2*2*) differ by only one amino acid residue, Arg-47 in the β1 subunit substituted with His-47 in the β2 subunit. ADH2 functions as a dimer and the β2β2 dimer exhibits about 100 times more catalytic activity than the β1β1 dimer.[1]

We previously reported on the influence of the *ADH2* and aldehyde dehydrogenase 2 genotypes on diabetic vasculopathy in type 2 diabetes.[2] Here we examined whether the *ADH2* genotype would also be associated with vascular events in community-dwelling Japanese and show the association of the *ADH2*1* allele with cerebral infarction.

Materials and methods. A population-based prospective cohort study of aging and age-related diseases was begun in Japan in 1997. All participants (1,126 men and 1,106 women) were independent residents of Aichi prefecture. Residents aged 40 to 79 years old were randomly selected from the register in cooperation with the local government. A total of over 1,000 characteristics, including medication, food and nutrition, bone mineral density, blood and urine analysis, psychological examinations, visual and auditory examinations, physical function tests and physical activities, anthropometry and body composition, and head MRI, were examined (see http://www.nils.go.jp/index-j.html).[3] The study protocol was approved by the Committee on the Ethics of Human Research of National Chubu Hospital and the National Center for Geriatrics and Gerontology. Written informed consent for the entire procedure was obtained from each participant.

Samples of DNA were isolated from peripheral blood cells. Genotypes were determined with a fluorescence-based allele-specific DNA primer-probe assay system (Toyobo Gene Analysis, Tsuruga, Japan). Brain MRI was performed using a 1.5-tesla scanner (Toshiba Visart, Tokyo). The first scanning sequence consisted of a T1-weighted sagittal series centered in the midline to define the orbitomeatal line. The second series of T1-weighted axial images and T2-weighted axial images were oriented parallel to the orbitomeatal line. Fourteen slices were taken at each examination.

A cerebral infarction was defined as a lesion more than 0.3 cm in diameter appearing as a low-signal-intensity area on T1-weighted images that was also visible as a hyperintense lesion on T2-weighted images as described.[3,4] Small lesions (<1.5 cm) were diagnosed as a lacunae. One of the authors (M.F.), a neurologist, who was blinded to the clinical status of the subjects, interpreted all MRI series.

Results. When the subjects were grouped into three according to the genotype of *ADH2*, *ADH2*2/ADH2*2 (ADH2*2/2)*, *ADH2*2/ADH2*1 (ADH2*2/1)*, and *ADH2*1/ADH2*1 (ADH2*1/1)*, the distribution of the *ADH2* genotypes was in Hardy-Weinberg equilibrium. There was no significant difference in characteristics among the three genotypic groups in women (data are not shown). In contrast, in men, the level of total cholesterol (TC) and LDL-cholesterol (LDL-C) significantly differed between the *ADH2*2/2* and *ADH2*1/2* genotypic groups by multiple comparisons (table 1). Although group *ADH2*1/1* did not significantly differ in the levels of TC and LDL-C from the other groups, probably due to an insufficient number in members of group *ADH2*1/1* (5.2%), the *ADH2*1* allele tended to increase the levels of TC and LDL-C. Additionally, alcohol consumption was higher in the *ADH2*1/1* group than the other groups, whereas there was no differ-

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Supported by a grant from the Ministry of Health, Labor and Welfare, Japan, to H.S. and S.O.

Received March 25, 2004. Accepted in final form June 24, 2004.

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*Table 1 Comparison of clinical characteristics in men among ADH2*2/2, ADH2*2/1, and ADH2*1/1 genotypic groups*

	*ADH2*2/2*	*ADH2*2/1*	*ADH2*1/1*	Genotype: *p* value
No. (%)	689 (61.2)	378 (33.6)	59 (5.2)	NS
Age, y	59.4 ± 0.4	58.8 ± 0.6	58.0 ± 1.4	NS
Alcohol, g/d	28.8 ± 1.4	29.5 ± 1.9	44.5 ± 4.5	2/2 vs 1/1: $p = 0.0049$*
				2/1 vs 1/1: $p = 0.0102$*
Nonsmoker & smoker, %†	21/40/39	22/40/37	24/39/37	NS
Systolic BP, mm Hg‡	120.1 ± 0.8	121.8 ± 1.0	126.1 ± 2.6	NS
Diastolic BP, mm Hg‡	74.9 ± 0.5	76.1 ± 0.6	77.3 ± 1.6	NS
Percent with hypertension§	32.6	37.0	40.7	NS
Height, cm	164.4 ± 0.2	164.7 ± 0.3	164.6 ± 0.8	NS
BMI	23.0 ± 0.1	22.8 ± 0.1	22.9 ± 0.4	NS
T-cho, mg/dL	210.1 ± 1.3	215.7 ± 1.7	217.6 ± 4.3	2/2 vs 2/1: $p = 0.0231$*
LDL, mg/dL	129.7 ± 1.2	135.8 ± 1.7	134.4 ± 4.2	2/2 vs 2/1: $p = 0.0115$*
HDL, mg/dL	57.3 ± 0.6	57.6 ± 0.8	57.4 ± 1.9	NS
TG, mg/dL	134.9 ± 3.7	130.8 ± 5.0	150.2 ± 12.4	NS
Glucose, mg/dL	105.7 ± 0.9	106.1 ± 1.2	103.9 ± 2.9	NS
HbA1c, %	5.32 ± 0.03	5.34 ± 0.04	5.33 ± 0.10	NS
Percent with diabetes	13.3	13.3	13.6	NS
Insulin, μU/mL	8.5 ± 0.2	7.8 ± 0.3	8.7 ± 0.7	NS
Estradiol, pg/mL	28.2 ± 0.4	27.1 ± 0.5	25.9 ± 1.4	NS
F-Testosterone, pg/mL	13.1 ± 0.2	13.3 ± 0.2	13.6 ± 0.5	NS
Brain examination, n (%)	n = 678	n = 367	n = 57	
Lacunal infarction	60 (8.9)	55 (15.0)	8 (14.0)	$p = 0.0085$¶
				2/2 vs 2/1: $p = 0.0025$‖
Cerebral infarction	68 (10.0)	59 (16.1)	9 (15.8)	$p = 0.0129$¶
				2/2 vs 2/1: $p = 0.0043$‖

Values are mean ± SD or n (%).

* *p* Value obtained by the Turkey-Kramer method for multiple comparisons.
† Nonsmoker & smoker = percentage of complete nonsmokers/percentage of past smokers who stopped smoking/percentage of current smokers.
‡ Blood pressure (BP) was analyzed only with subjects not taking oral antihypertension medications.
§ Hypertension was defined as either a systolic blood pressure of over 140 mm Hg or a diastolic blood pressure of over 90 mm Hg, or as receiving antihypertension medication.
¶ *p* Value obtained by the contingency table analysis.
‖ *p* Value by the chi-square analysis between groups *ADH2*2/2* and *ADH2*2/1*.

NS = not significant by multiple comparisons; BMI = body mass index; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

ence in amounts of alcohol consumption between groups *ADH2*2/2* and *ADH2*2/1*.

A total of 1,102 male and 1,093 female subjects were examined by MRI. More striking, in men, higher frequencies of lacunae and cerebral infarction were found in the *ADH2*2/1* group than the *ADH2*2/2* group (see table 1). The frequencies of other abnormal signs on MRI did not differ among the three groups (data are not shown). In women, there was no difference in prevalence of abnormal MRI signs among the three *ADH2* genotypic groups (data not shown).

To confirm the significant difference in the frequencies of lacunae and cerebral infarction according to the *ADH2* genotype, multiple logistic analyses were performed based on 1,102 subjects with an adjustment for aging (table 2). Aging is the most significant risk for lacunae and cerebral infarction. More interestingly, OR and *p* values clearly indicated that the *ADH2*1* allele is a distinct risk for lacunae and cerebral infarction. Even when the effect of alcohol consumption was included, the main conclusion was not altered (see table 2).

Discussion. An influence on lacunae and cerebral infarction by the *ADH* genotype was found only in Japanese men. This discrepancy between genders may be speculated to be due to a difference in alcohol consumption. However, even when the effect of alcohol consumption was included, the main conclusion was not altered. Therefore, the effect by alcohol consumption does not seem responsible for the discrepancy between genders. Instead, ADH2 activity modulated by several hormones may be responsible for the discrepancy. In fact, experiments with ani-

Table 2 Multiple logistic analyses (number of subjects = 1,102)

	OR (95% CI)	*p* Value
Lacunar state in men		
A: Multiple logistic analyses		
ADH2 (carriage of *ADH2*1* allele)	2.16 (1.44–3.25)	0.0002
Age - 10 y	3.46 (2.69–4.45)	<0.0001
B: Multiple logistic analyses including alcohol consumption		
ADH2 (carriage of *ADH2*1* allele)	2.18 (1.49–3.38)	0.0005
Age - 10 y	3.53 (2.68–4.65)	<0.0001
Cerebral infarction in men		
A: Multiple logistic analyses		
ADH2 (carriage of *ADH2*1* allele)	2.06 (1.39–3.06)	0.0003
Age - 10 y	3.44 (2.70–4.37)	<0.0001
B: Multiple logistic analyses including alcohol consumption		
ADH2 (carriage of *ADH2*1* allele)	2.05 (1.35–3.11)	0.0008
Age - 10 y	3.49 (2.70–4.52)	<0.0001

mals indicated that testosterone reduces enzymatic activity in the liver, and that estrogen increases the activity.[5]

ADH catalyzed the first step in the metabolism of ethanol, and in addition, has a wide substrate range, using both aliphatic and aromatic alcohols, aldehydes, sterols, and ω-hydroxy fatty acids. It is worth noting that ADH catalyzes the oxidation of 3,3-dimethylallyl alcohol, the intermediary alcohol of the shunt pathway of mevalonate metabolism, and the branching between the sterol and the shunt pathway could also occur at the level of geranyl pyrophosphate and farnesyl pyrophosphate.[6] Therefore, the genetic variant of *ADH2* may change the flow of the shunt pathway of cholesterol synthesis, thereby causing LDL-C levels to vary between the *ADH2*2/2* and *ADH2*2/1* groups. As for cardiovascular diseases, it was reported that an ADH3 polymorphism is associated with HDL-C levels and myocardial infarction in Caucasians.[7] Thus, our results may provide insight into ethnic differences in the incidence of cerebral or myocardial vascular disease between Mongoloids and Caucasians.

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Geriatrics and Gerontology International 2004; **4:** *S289–S291*

Interactions between health and psychological changes in Japanese: the NILS-LSA

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A comprehensive longitudinal study would be essential in the analyses of psychological changes. At the National Institute for Longevity Sciences (NILS), a comprehensive longitudinal study of aging in Japan, the NILS Longitudinal Study of Aging (NILS-LSA) started in November 1997. The participants of this study were 2300 residents aged 40–79 years who were random samples selected from the neighborhood area of the NILS. They were examined every 2 years at the NILS-LSA Examination Center. From the recent results of the NILS-LSA, interactions between health and psychological changes including mental effects of disease, relationship between physical health and cognitive function, and association of depression with nutrition and physical activity were shown.

Keywords: longitudinal studies, psychology, health, aging, epidemiology.

NILS-LSA: National Institute for Longevity Sciences Longitudinal Study of Aging

Aging and health are strongly associated with psychological changes including cognitive function, depression, anxiety, self-esteem, personality, and quality of life (QOL). In the study of psychological changes in the elderly, various health-related factors such as medical problems, physical health, lifestyle, physical activity, nutrition, smoking, and alcohol should be assessed, and effects of these health-related factors on the psychological changes should be analyzed longitudinally. Thus, a comprehensive longitudinal study would be essential in the analyses of individual psychological changes.

In 1995, a national research institute of aging in Japan, the National Institute for Longevity Sciences (NILS) was established, and in 1997, a comprehensive study of aging and geriatrics, the NILS Longitudinal Study of Aging (NILS-LSA) commenced. The main purpose of the NILS-LSA is to describe the physiological and psychological process in aging. The NILS-LSA also aims to assess the effects of lifestyle, stress, and disease on aging, to detect early markers of disease and disability, to determine normal range of indices of aging, to separate disease from aging, and to determine biological aging. Subjects were male and female residents aged 40–79 years who were randomly selected from the neighborhood area of the NILS. Selected males and females who were assigned to the examination were invited by mail to an explanatory meeting. At the explanatory meeting, procedures for each examination and the importance of continuation to follow-up were fully explained. Participants were limited to those who accepted examination procedures and signed their names on a written form. Everyday, seven participants were examined from 08.30 hours to 17.00 hours at a special examination center (Fig. 1). The first-wave examination commenced in November 1997 and finished in April 2000. Two thousand two hundred and sixty-seven participants were examined, and they have been examined every subsequent 2 years. The second-wave examination started in 2000, and the third-wave examination started in 2002 (Fig. 2). Observed variables were: (1) past and present history, and familial history of geriatric disease; (2) lifestyle and environment; (3) medical examinations of geriatric diseases including head MRI, cardiovascular functions, bone mineral density, body fat, and body water; (4) nutritional assessments by food frequency questionnaire and dietary diary; (5) physical activities and physical functions; and (6) psychological assessments such as personality, cognition, emotion, social adaptation, and life-events. Using these

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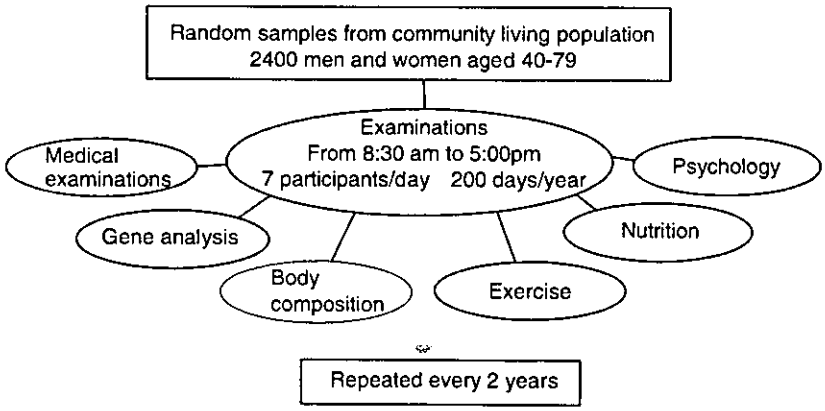

Figure 1 National Institute for Longevity Sciences, Longitudinal Study of Aging (NILS-LSA).

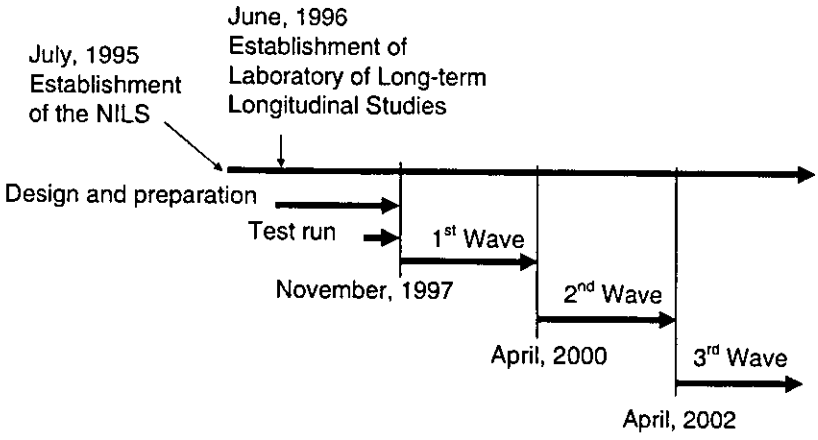

Figure 2 Development of the NILS-LSA.

variables, the relationship between health and psychological changes were analyzed.

Experience of health problems and everyday activities

Age difference in impact of health problems, such as disease or injury, on everyday activities and depressive symptoms were examined in the participants of the NILS-LSA. How the type and source of social interactions moderated the noxious effects of health problems was also examined. Everyday activities were measured using the Tokyo Metropolitan Institute of Gerontology Index of Competence (TMIG-IC) and depressive symptoms were assessed with the Center for Epidemiologic Studies Depression Scale (CES-D). Longitudinal analyses of the NILS-LSA data indicated that health problems were significantly related to (a) an increase in depressive symptoms among middle-aged adults, and (b) a decline in everyday activities among older adults. The former (a) was buffered by emotional family support, whereas the latter (b) was buffered by instrumental family support and surprisingly, by negative interactions with family. In contrast, social interactions with other friends and acquaintances did not show any moderating effect.

Physical activity and depression

The antidepressant effect of physical activity has been of increasing interest in recent years. Several studies have indicated that the benefits of exercise are not restricted to experimental studies for moderately or clinically depressed persons. The associations between physical activity and depressive symptoms in the participants of the NILS-LSA were examined. Physical activity was measured using a pedometer whereas depressive symptoms were assessed with CES-D. Cross-lagged longitudinal analyses using structural equation modeling revealed that, for the older adults (aged 65–79 years), daily walking at baseline predicted less depressive symptoms at the 2-year follow-up, even after adjusting for confounders. In contrast, the association was not confirmed for the mid-life adults (aged 40–64 years). Findings suggest that age should be considered when the effect of activity on psychological wellbeing is estimated.

Dietary cholesterol and depression

Some studies have suggested that low serum cholesterol induced by medication increases the incidence of suicides. A few studies also mentioned that low serum cho-

lesterol concentration by diet induces depression. The relationship of dietary cholesterol intake and serum total cholesterol level with depression was examined in the NILS-LSA participants. The mean depression score (CES-D) in the lower third of dietary cholesterol was significantly higher than that of the middle or higher third in males and the trend remained even after adjusting for total energy intake. However, the serum cholesterol level did not relate significantly with CES-D scores. The prevalence of depression in the lower dietary cholesterol third was significantly higher than that in the middle or higher third in males. Even in females, the prevalence increased with the decrease of cholesterol intake. The subjects were divided into the thirds according to their total energy intakes. In the lower energy intake group, the prevalence of depression in the lower dietary cholesterol third was remarkably high (22.1% in males and 31.0% in females).

Health, lifestyle, gene, and cognitive function

A head MRI is taken for the each NILS-LSA participant and stored in an image database. Intracranial tumors and vascular lesions are checked, and brain volume is estimated via a computerized trace of the MRI. Cognitive function is assessed by IQ levels determined by WAIS-R-SF in all participants. In addition to the IQ levels, MMSE is also used for the assessment in the participants aged 60 years and over. Results from the assessment of cognitive function showed that 5.9% of the participants aged 60 years or over had cognitive impairment. The relationship between cognitive function and various health-related variables were assessed. Aging, pathological changes of brain, smoking, alcohol, physical activity, depression, and glucose metabolism were significantly related to cognitive decline.

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特別講演

高齢者の健康と栄養

下　方　浩　史

1．日本人はなぜ長生きか

2003年度のWHOの報告によると，日本人の平均寿命は世界192カ国中で一番長く81.9歳に達している。日本人の平均寿命がなぜ長いのか，この問いに対する明確な答えは今のところ出されていない。ここではいくつかの可能性のある長寿要因を述べてみる[1]。

まず，日本における医療制度の充実と社会的な長寿要因の存在である。日本人の乳幼児の死亡率は諸外国に較べて低い。小児医療が充実しており，乳幼児の健康が，そして生命が手厚く守られている。また，国民皆保険制度の存在や高齢者に対する医療制度が比較的整備されていることも重要であろう。老人検診などの健康診断も広く実施されて，健康増進や病気の早期発見，早期治療につながっている。

日本人は高齢になっても勤労意欲が高く，また，実際に社会参加率が高い。高齢者の社会参加が寿命の延長につながっているということを示す研究結果も出されている。日本の社会が比較的平等で，貧富の差が少ないことも長寿要因となっているかもしれない。米国のような自由競争社会では劣悪な健康状態を強いられている貧困層が存在し，国民全体の平均寿命を短くしている。また，日本では諸外国に較べ学校教育が充実している。教育によって国民全体の健康に関する知識や関心が高まっていると思われる。

日本人の食事や運動，入浴などのライフスタイルが長寿に適していることも考えられている。日本には独特の食習慣がある。先進諸国中で脂肪摂取量が飛び抜けて少なく，米飯を中心として炭水化物の摂取が多い。また，魚の摂取が多いことも特徴である。豆腐や納豆，味噌などの大豆製品の摂取が多く，これらは動脈硬化の進行を防ぐには理想に近い食習慣である。また，カテキンやビタミンCなどの抗酸化物質が多く含まれる緑茶の摂取は，動脈硬化や癌を防いでいる可能性がある。高齢になっても社会参加を続けていることで運動量を保つことが出来ている。清潔好きも重要な要因であろう。毎日入浴し，身の回りを常に清潔に保っている。このことが感染症の予防につながっていると推測される。

この他にも遺伝的素因などの影響もあるが，ここでは長寿に特に重要だと思われる栄養について，長寿や高齢者の健康に関連して述べてみる。

2．理想的肥満度

食餌制限と寿命との関係については，1930年代のMcCayによるラットを使った有名な

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