Table 2
The levels of arsenic in toenail samples.

As	*N*	Mean ppm*	[95% Conf. Interval]	
Total	159	0.41	0.36	0.47
Male	52	0.42	0.35	0.51
Female	107	0.41	0.34	0.48
p value				0.765
Age (in years)				
60–69	65	0.42	0.33	0.53
70–79	71	0.40	0.34	0.46
80+	23	0.42	0.28	0.62
p for trend				0.881

*geometric mean
p value was obtained by univariate linear regression analysis.

rice or more everyday was higher than those consuming 3 bowls or less ($p<0.001$, univariate analysis). The association between rice consumption and arsenic levels was significant after adjustment for smoking. We did not find sex differences between toenail arsenic levels and dietary habits. Table 7 lists the five subjects consuming 4 bowls of rice or more a day. No particular demographic characteristics or other factors were noted among them.

4. Discussion

In the present study, the geometric mean level of toenail total arsenic was 0.41 ppm among participants of health check-up surveys conducted in a town of Amami-Oshima Island. The maximum and minimum arsenic levels were 13.75 and 0.15 ppm, respectively. We did not measure arsenic levels according to chemical forms. According to a National Academy of Sciences report on the Medical and Biological Effect of Environmental Pollutants[7] the amount of arsenic in toenail is usually from 0.43 to 1.08 ppm. There were 5 males and 6 females who had arsenic concentration values higher than 1.08 ppm in the present study. The geometric mean level is about 3-fold higher than the geometric mean level we obtained in a study conducted in mainland Kagoshima, where toenail arsenic levels were 0.15 ppm on average among 57 adult male residents (Fig. 1). Table 8 summarizes the arsenic levels in hair and toenail samples, reported by domestic and international studies, including the present one. Japanese arsenic levels were between those of Pakistan and The United States.

Many studies reported sex differences in hair and toenail arsenic levels. For example, hair and toenail arsenic concentrations were higher in males than females.[10,13–15] Interestingly, a study reported that arsenic content in fingernails was higher in females than in males.[10] On the other hand, several studies showed no evident sex difference in hair and toenail arsenic levels.[8,16] The present study showed no evident sex difference in toenail arsenic levels (males = 0.42 ppm, female = 0.41 ppm) either.

In the present study, arsenic levels were elevated among current smokers ($p<0.001$). There was a relationship between the number of cigarettes smoked per day and toenail

Table 3
The levels of arsenic in toenail samples according to smoking status.

	N	Mean ppm*	[95% Conf. Interval]	
Male and Female				
never smoked	88	0.41	0.34	0.50
ex-smoker	22	0.35	0.31	0.39
current-smoker	13	0.65	0.32	1.29
unknown	36	0.38	0.32	0.46
p for trend[1]				0.279
p for trend[2]				0.190
Male				
never smoked	11	0.31	0.25	0.37
ex-smoker	21	0.36	0.32	0.39
current-smoker	10	0.87	0.39	1.96
unknown	10	0.41	0.26	0.66
p for trend[1]				<0.001
Female				
never smoked	77	0.43	0.35	0.54
ex-smoker	1	0.21	–	–
current-smoker	3	0.24	0.08	0.70
unknown	26	0.37	0.30	0.45
p for trend[1]				0.229
Male and Female				
non-smoker	110	0.40	0.34	0.47
current-smoker	13	0.65	0.32	1.29
unknown	36	0.38	0.32	0.46
p for trend[1]				0.059
p for trend[2]				0.051
Male				
non-smoker	32	0.34	0.31	0.37
current-smoker	10	0.87	0.39	1.96
unknown	10	0.41	0.26	0.66
p for trend[1]				<0.001
Female				
non-smoker	78	0.43	0.34	0.53
current-smoker	3	0.24	0.08	0.70
unknown	26	0.37	0.30	0.45
p for trend[1]				0.303

*geometric mean.
[1]*p* value was obtained by univariate linear regression analysis.
[2]*p* value was obtained by mulutivariate linear regression analysis for sex and age.

Table 4
The levels of arsenic in toenail samples and the number of cigarettes per day in males.

Tobacco/day	*N*	Mean ppm*	[95% Conf. Interval]	
0 (never + ex-smoker)	32	0.34	0.31	0.37
1–15	4	1.36	0.16	11.55
16–25	5	0.48	0.21	1.08
26+	1	3.15	–	–
p for trend[1]				0.001

*geometric mean.
[1]*p* value was obtained by univariate linear regression analysis.

Table 5
The levels of arsenic in toenail samples according to drinking status.

	N	Mean ppm*	[95% Conf. Interval]	
Male and Female				
never drank	92	0.41	0.34	0.50
ex-drinker	4	0.81	0.08	7.86
sometimes	13	0.44	0.29	0.67
everyday	14	0.37	0.25	0.57
unknown	36	0.38	0.32	0.46
p for trend[1]				0.907
p for trend[2]				0.844
Male				
never drank	16	0.33	0.30	0.37
ex-drinker	3	1.04	0.02	61.21
sometimes	12	0.47	0.30	0.72
everyday	11	0.43	0.26	0.71
unknown	10	0.41	0.26	0.66
p for trend[1]				0.363
Female				
never drank	76	0.43	0.35	0.54
ex-drinker	1	0.39	–	–
sometimes	1	0.21	–	–
everyday	3	0.23	0.09	0.57
unknown	26	0.37	0.30	0.45
p for trend[1]				0.187

*geometric mean.
[1]*p* value was obtained by univariate linear regression analysis.
[2]*p* value was obtained by mulutivariate linear regression analysis for sex and age.

Table 6
The levels of arsenic in toenail samples according to foods consumption.

	N	Mean ppm*	[95% Conf. Interval]	
Fish				
1–2 times/month	6	0.37	0.25	0.57
1–2 times/week	21	0.48	0.29	0.81
3–4 times/week	49	0.39	0.32	0.47
everyday	47	0.43	0.32	0.58
p for trend[1]				0.996
p for trend[2]				0.961
Seaweed				
< 1 time/week	7	0.41	0.20	0.88
1–2 time/week	12	0.34	0.25	0.48
3–4 time/week	30	0.38	0.31	0.45
every day	73	0.46	0.36	0.58
p for trend[1]				0.310
p for trend[2]				0.295
Rice				
1 bowls**/day	7	0.56	0.18	1.71
2 bowls**/day	26	0.31	0.24	0.39
3 bowls**/day	85	0.41	0.35	0.49
> 4 bowls**/day	5	1.97	0.25	15.75
p for trend[1]				0.050
p for trend[2]				0.052

*geometric mean.
**1bowl is about 150 g.
[1]*p* value was obtained by univariate linear regression analysis.
[2]*p* value was obtained by mulutivariate linear regression analysis for sex and age.

Table7
Characteristics of residents consuming 4 bowls of rice or more everyday.

Rice (bowls**/day)	Arsenic conc***	Sex	Age (in years)	Smoking	Alcohol
4	6.83	male	78	current-smoker	ex-drinker
4	0.35	female	78	current-smoker	never drank
5	0.36	male	69	ex-smoker	everyday
6	2.48	male	66	current-smoker	sometimes
6	13.75	female	69	never smoked	never drank

**1bowl is about 150 g.
***The level of arsenic in toenail (ppm).

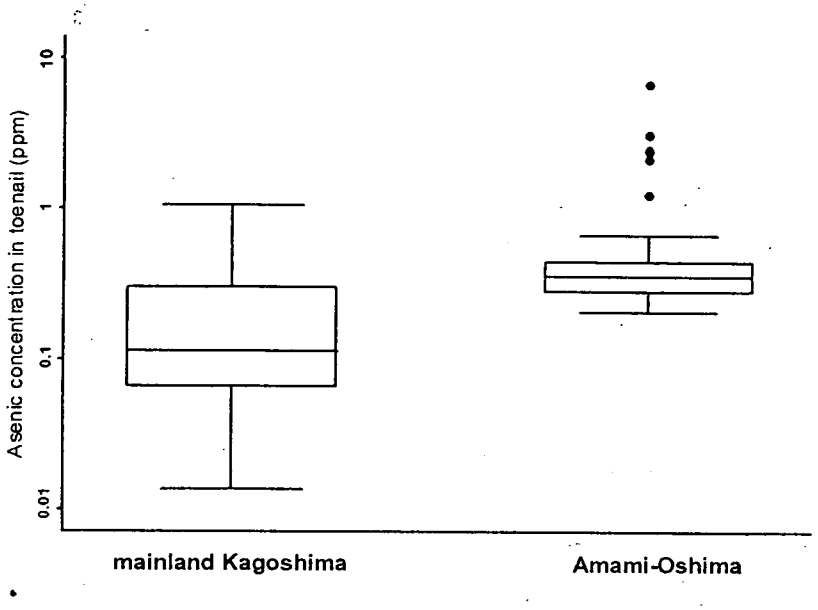

Fig. 1. The levels of arsenic in toenail samples.

Table 8
Hair and toenail arsenic levels in various populations.

Hair				Toenails		
Country	*N*	ppm	Reference	*N*	ppm	Reference
Arsenic						
Pakistan	160	0.31*	Anwar, M., 2005[8]	160	0.69*	Anwar, M., 2005[8]
The U. S.	55	0.02	Takagi,Y. *et al.*,1986[2]	117	0.031	Vance, D.E. *et al.*1988[9]
Japan	457	0.05	Takagi,Y. *et al.*,1986[2]			
Japan				157	0.41*	Present study in Amami-Oshima Island
Japan				57	0.15*	Present study in mainland Kagoshima
India	255	0.61	Takagi,Y. *et al.*,1986[2]			
Canada	92	0.016	Takagi,Y. *et al.*,1986[2]			
Poland	46	0.02	Takagi,Y. *et al.*,1986[2]			
Malaysia	NA	0.28	Oluwole, *et al.*,1994[25]			
Nigeria	100	0.09	Oluwole, *et al.*,1990[26]			
Italy	NA	0.09	Caroli, *et al.*,1992[27]			

*geometric means.

arsenic levels in males. Toenail arsenic levels did not show any evident difference between ex-smokers and non-smokers. Moreover, there was no relationship between arsenic concentration and number of years since quitting smoking, suggesting that the effect of smoking on arsenic levels did not last long. Passive smoking was not examined in this study. The present study is not the first to find a relationship between smoking and arsenic levels. Saad and Hassanien found that smoking was related to hair arsenic levels in an Egyptian population without any known occupational exposure to arsenic.[16] Wolfsperger *et al.* also compared arsenic levels of hair samples in smokers and non-smokers in Austria and Italy, and reported geometric means to be 0.081 ppm and 0.065 ppm in smokers and non-smokers, respectively. However, the observed difference was not statistically significant.[13] It is known that arsenic is contained in tobacco and the arsenic concentration in tobacco has been decreasing since the 1960s. The arsenic concentration of Japanese tobacco is estimated to be 1 mg/kg or less in recent years, and the amount of arsenic inhaled by the average smoker is 0.02 mg or less.[17] We measured the arsenic concentrations of 10 popular Japanese cigarettes. The mean arsenic level was 0.19 ppm (95% confidence interval, 0.15–0.22). In terms of the brand of tobacco, there were no large differences in the concentrations of arsenic.

We found significant correlations between the levels of arsenic and the following trace elements: manganese (correlation coefficient = 0.15 and p value = 0.004), lead (0.14, p = 0.024), selenium (0.75, p = 0.001), strontium (0.20, p = 0.009), vanadium (0.18, p = 0.002) and zinc (0.61, p = 0.042). On the other hand, arsenic levels were not related to the following trace elements: cadmium (correlation coefficient = 0.03 and p value = 0.685), copper (0.31, p = 0.060), or cobalt (0.092, p = 0.092). It is of note that arsenic levels were strongly associated with the levels of zinc and selenium, which are known to be contained at high levels in seaweed and fish. [18,19] Since arsenic in fish meat is in the form of trimethyl arsenic, which is known to tend not to accumulate in hair and toenail, our data suggest that seaweeds, rather than fish, may be the major source of those elements in toenail samples. Although our study did not show any significant differences between seaweed consumption and arsenic levels in toenail, dietary surveys are well-known for its difficulty in collecting accurate information. Indeed, we had an impression that seaweed consumption given by some respondents did not include seaweeds collected at the near by seashore.

The Canadian Food Inspection Agency and the UK Food Standards Agency, which conducted surveys of arsenic in a variety of seaweeds, and found that hijiki contained a high level of inorganic arsenic, advised people to avoid the consumption of hijiki seaweed.[20,21] In the present study, there were only 3 persons who ate hijiki. The arsenic levels were determined in 2 of those 3 subjects, and the levels were 0.39 ppm and 0.29 ppm, which were lower than the average in the town in Amami-Oshima Island. The majority (148 persons) of our study subjects routinely consumed wakame seaweed. Wakame consumption was not related to toenail arsenic levels.

Rice has higher inorganic arsenic concentrations than most other foods. Consequently, diets relying heavily on rice may contain high inorganic arsenic. A study showed the presence of four forms of inorganic arsenic in Arborio rice: inorganic arsenic (III), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), and inorganic arsenic (V). Their concentrations were 88.2+/–7.1, 50.8+/–5.0, 15.2+/–1.7, and 51.2+/–3.5 ng/g, respec-

tively.[22] In Japan, Ibaraki prefectural institute of public health measured rice arsenic levels (0.06–0.17 ppm) at 18 places of production in 2003. In the present study, we found a positive relationship between rice intake and arsenic levels ($p = 0.050$). Although the mean toenail arsenic level in our study area in Amami-Oshima Island was about 3-fold higher than that observed in mainland Kagoshima, the area differences in the amounts of rice consumption and/or arsenic concentration in rice are unlikely to explain the area difference in toe-nail arsenic concentrations. We could not find any reports on the relationship between rice contamination and arsenic in toenails and/or hair in the literature. Serious arsenic contamination of the rice in India and Bangladesh has been reported.[23,24] It was reported that arsenic contamination of the rice in Bangladesh and India was based on the groundwater at the time of cultivation and cooking. In the present study, we did not measure arsenic contamination of the water. However we usually use tap water. The arsenic action level in Japanese tap water is decided to be 0.01 ppm or less.

In conclusion, the present study indicated arsenic exposure levels in Amami-Oshima Island might be about 3-fold higher than those in mainland Kagoshima. Among various lifestyles, smoking among men, and rice consumption in general were related to arsenic levels. However, it is unlikely that smoking or rice consumption can explain. Although some seaweeds are known to contain high levels of arsenic, we could not find a correlation between seaweed consumption and toenail arsenic levels. Further studies seem warranted to detemine the cause of the relatively high arsenic exposure levels in the study area.

References

1 Arsenic contamination in ground water affecting some countries in South-East Asia region. (2001): REGIONAL COMMITTEE Provisional Agenda item 9. Fifty-fourth SessionSEA/RC54/83.

2 Takagi, Y., Matsuda, S., Imai, S., Ohmori, Y., Masuda, T., Vinson, J.A., Mehra, M.C., Puri, B.K. and Kaniewski, A. (1986): Trace elements in human hair: an international comparison. *Bull Environ Contam. Toxicol.* **36(6)**: 793–800.

3 Gunderson, E.L. (1995): FDA total diet study, July 1986–April 1991, dietary intakes of pesticides, selected elements, and other chemicals. *J. AOAC Int.* **78**: 1353–1363.

4 Dabeka, R.W., McKenzie, A.D., Lacroix, G.M.A., Cleroux, C., Bowe, S., Graham, R.A., Conacher, H.B.S. and Verdier, P. (1993): Survey of arsenic in total diet food composites and estimation of the dietary intake of arsenic by Canadian adults and children. *J. AOAC Int.* **76**: 14–25.

5 Tsuda, T., Inoue, T., Kojima, M. and Shigeru, A. (1995): Market basket and duplicate portion estimation of dietary intakes of cadmium, mercury, arsenic, copper, manganese, and zinc by Japanese adults. *J. AOAC Int.* **78**: 1363–1368.

6 Schoof, R.A., Yost, L.J., Eickhoff, J., Crecelius, E.A., Cragin, D.W., Meacher, D.M. and Menzel, D.B. (1999): A market basket survey of inorganic arsenic in food. *Food Chem Toxicol.* **37(8)**: 839–846.

7 National Academy of Sciences, Washington, D.C., U.S.A. (1977): *Medical and Biologic Effects of Environmental Pollutants* **16.**

8 Anwar, M. (2005): Arsenic, cadmium and lead levels in hair and toenail samples in Pakistan. *Environ. Sci.* **12(2)**: 71–86.

9 Vance, D.E., Ehmann, W.D. and Markesbery, W.R. (1988): Trace element content in fingernails and hair of a non-industrialized US control population. *Biol. Trace Elem. Res.* **17**: 109–121.

10 Mandal, B.K., Ogra, Y. and Suzuki, K.T. (2003): Speciation of arsenic in human nail and hair from arsenic-affected area by HPLC-inductively coupled argon plasma mass spectrometry. *Toxicol. Appl. Pharmacol.* **189(2)**: 73–83.
11 Nowak, B. and Kozlowski, H. (1998): Heavy metals in human hair and teeth: the correlation with metal concentration in the environment. *Biol. Trace Elem. Res.* **62(3)**: 213–228.
12 Yoshinaga, J., Imai, H., Nakazawa, M., Suzuki, T. and Morita, M. (1990): Lack of significantly positive correlations between elemental concentrations in hair and in organs. *Sci. Total Environ.* **99(1–2)**: 125–135.
13 Wolfsperger, M., Hauser, G., Gossler, W. and Schlagenhaufen, C. (1994): Heavy metals in human hair samples from Austria and Italy: influence of sex and smoking habits. *Sci. Total Environ.* **156(3)**: 235–242.
14 Chiou, H.Y., Hsueh, Y.M., Hsieh, L.L., Hsu, L.I., Hsu, Y.H., Hsieh, F.I., Wei, M.L., Chen, H.C., Yang, H.T., Leu, L.C., Chu, T.H., Chen-Wu, C., Yang, M.H. and Chen, C.J. (1997): Arsenic methylation capacity, body retention, and null genotypes of glutathione S-transferase M1 and T1 among current arsenic-exposed residents in Taiwan. *Mutat. Res.* **386(3)**: 197–207.
15 Rogers, C.E., Tomita, A.V., Trowbridge, P.R., Gone, J.K., Chen, J., Zeeb, P., Hemond, H.F., Thilly, W.G., Olmez, I. and Durant, J.L. (1997): Hair analysis does not support hypothesized arsenic and chromium exposure from drinking water in Woburn, Massachusetts. *Environ. Health Perspect.* **105(10)**: 1090–1097.
16 Saad, A. and Hassanien, M.A. (2001): Assessment of arsenic level in the hair of the nonoccupational Egyptian population. *Environ. Int.* **6**: 471–478.
17 WHO (1983): *Environmental Health Criteria* **18**: 1–174.
18 Shibata, Y., Morita, M. and Fuwa, K. (1992): Selenium and arsenic in biology: their chemical forms and biological functions. *Adv. Biophys.* **28**: 31–80.
19 Volesky, B. and Holan, Z.R. (1995): Biosorption of heavy metals. *Biotechnol. Prog.* **11(3)**: 235–250.
20 Katayama, M., Katayama, Y.S., Sakiyama, C., Kouya, Y., Furukawa, C. and Hanaoka, K. (2002): Arsenic Metabolites Excreted into the Human Urine after Hijiki Intake. The 19th Symposium on Trace Nutrients Research: pp. 27–33.
21 DiPietro, E.S., Phillips, D.L., Paschal, D.C. and Neese, J.W. (1989): Determination of trace elements in human hair. Reference intervals for 28 elements in nonoccupationally exposed adults in the US and effects of hair treatments. *Biol. Trace Elem. Res.* **22(1)**: 83–100.
22 D'Amato, M., Forte, G. and Caroli, S. (2004): Identification and quantification of major species of arsenic in rice. *J. AOAC Int.* **87(1)**: 238–243.
23 Bae, M., Watanabe, C., Inaoka, T., Sekiyama, M., Sudo, N., Bokul, M.H. and Ohtsuka, R. (2002): Arsenic in cooked rice in Bangladesh. *Lancet* **360(9348)**: 1839–40. Erratum in: *Lancet* **361(9362)**: 1060.
24 Patel, K.S., Shrivas, K., Brandt, R., Jakubowski, N., Corns, W., Hoffmann, P. (2005): Arsenic contamination in water, soil, sediment and rice of central India. *Environ. Geochem. Health* **27(2)**: 131–145.
25 Oluwole, A.F., Ojo, J.O., Durosinmi, M.A., Asubiojo, O.I., Akanle, O.A., Spyrou, N.M. and Filby, R.H. (1994): Composition of head hair and fingernails of some Nigerian subjects. *Biol. Trace Elem. Res.* **43–45**: 443–452.
26 Oluwole, A.F., Asubiojo, O.I., Adekile, A.D., Filby, R.H., Bragg, A. and Grimm, C.I. (1990): Trace element distribution in the hair of some sickle cell anemia patients and controls. *Biol. Trace Elem. Res.* **26–27**:479–484.
27 Caroli, S., Senofonte, O., Violante, N., Fornarelli, L. and Powar, A. (1992): Assessment of reference values for elements in hair of urban normal subjects. *Microchem. J.* **46**: 174–183.

Original Research Article

Dementia
and Geriatric
Cognitive Disorders

Dement Geriatr Cogn Disord 2006;22:8–14
DOI: 10.1159/000092958

Accepted: December 28, 2005
Published online: April 24, 2006

Microbleeds in Alzheimer Disease Are More Related to Cerebral Amyloid Angiopathy than Cerebrovascular Disease

Yuriko Nakata-Kudo[a] Toshiki Mizuno[a] Kei Yamada[b] Kensuke Shiga[a]
Kenji Yoshikawa[a] Satoru Mori[a] Tunehiko Nishimura[b] Kenji Nakajima[c]
Masanori Nakagawa[a]

Departments of [a]Neurology and [b]Radiology, Kyoto Prefectural University of Medicine, and
[c]Kyoto Interdisciplinary Institute of Community Medicine, Kyoto, Japan

Key Words
Cerebral amyloid angiopathy · Alzheimer's disease · Microbleeds · White matter lesions

Abstract
Cerebral amyloid angiopathy (CAA) is one of the cardinal pathological features in the vascular components of Alzheimer's disease (AD). CAA itself results in disrupted microvasculature, mainly in the cerebral cortex, eventually leading to a brain cortical or subcortical hemorrhage in a population of elderly people, but clinically overt brain hemorrhages are not so frequent in AD patients. Here we assessed 50 AD patients and 26 controls to detect latent brain hemorrhages with gradient-echo T_2*-weighted images, a sensitive magnetic resonance imaging technique to detect hemosiderin components in the brain. Microbleeds, demarcated as low-intensity spots in T_2*-weighted images, were detected in 16.7% of AD patients without cerebrovascular disease (CVD) and in 12.5% of those with CVD, while no microbleeding was detected in the control subjects. No significant difference was observed between the microbleed-positive group and the microbleed-negative counterpart in their clinical background, such as hypertension, the use of antiplatelet drugs and smoking. In addition, white matter high intensities in the T_2-weighted image were significantly more confluent in the microbleed-positive AD group than its negative counterpart. In conclusion, our evaluation of AD brains revealed that latent microbleeds in AD patients are more frequent than in normal controls. Microbleeds not being related to common hemorrhagic risk factors, but being significantly related to white matter pathologies suggested that microbleeds in AD may be associated with CAA, but not with hypertension or CVD.

Introduction

The central pathological features of Alzheimer's disease (AD) are a profound loss of neurons, senile plaques and neurofibrillary tangles, while cerebral amyloid angiopathy (CAA), the cerebral Aβ deposition in the leptomeningeal and intracortical vessels, is another important histopathological component in AD. The Aβ deposition in the small arteries is believed to cause a loss of smooth muscle cells in the medial and adventitial layers, eventually resulting in vascular dilatation and the development

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1420–8008/06/0221–0008$23.50/0

Accessible online at:
www.karger.com/dem

Dr. Toshiki Mizuno
Kyoto Prefectural University of Medicine, Department of Neurology
Kajiicho-465, Kamigyo-ku
Kyoto 602-8566 (Japan)
Tel. +81 75 251 5794, Fax +81 75 211 8645, E-Mail mizuno@koto.kpu-m.ac.jp

of microaneurysms in the cortex [1]. The collapse of such a microaneurysm leads to a cortical or subcortical (C/CS) hemorrhage, but clinically overt brain hemorrhages are not particularly frequent in AD patients [2].

The evaluation of CAA in AD has been limited to postmortem pathological exploration because most of the microbleeds in AD are asymptomatic and too small to be visualized in conventional computed tomography (CT) or magnetic resonance imaging (MRI) [3]. Deoxyhemoglobin in the brain hemorrhage produces a nonuniform magnetic field that results in the rapid dephasing of proton spins in T_2-weighted imaging, and more so in gradient-echo T_2*-weighted images. Therefore, hemosiderin-laden areas, even if they are small in volume, appear dark in T_2*-weighted images. Pathological studies have proved that such signal loss in T_2*-weighted images corresponds to a focal deposition of hemosiderin-laden macrophages in the brain [4].

Our prior studies [3] using T_2* revealed that microbleeds in the C/CS area in AD were found in 18.4%, which was much more frequent than in a previous pathological exploration that indicates 5.1% [5]. We were able to depict more lesions using T_2*-weighted images possibly because of the following two reasons. First, the T_2*-weighted images are able to exaggerate the existence of hemosiderin within the brain tissue, which will demonstrate a substantially larger area of signal abnormality than its real size. Secondly, the MRI study enables thinner slice section than brain cutting.

Hanyu et al. [6] also reported that microbleeds in the C/CS area were detected in 27.1% of the Japanese AD patients, which was substantially higher than the occurrence in their control subjects. Their population, however, included AD patients and control subjects with silent lacunar infarction. Therefore, T_2*-verified microbleeds in their study might not only include microbleeds from pure CAA [7], but also those from hemorrhagic [4] and ischemic cerebrovascular diseases (CVD) [8]. As the etiology of microbleeds in patients with AD will likely be different from the etiology of microbleeds in those with CVD, we thought that it would be important to compare the incidence of microbleeds between AD patients with CVD and those without CVD.

We therefore divided our AD patients into two groups (with or without CVD) for the analysis. In addition, the control subjects in our study were selected from those without CVD on imaging studies. We also assessed whether T_2*-verified microbleeds in AD might be related to white matter lesions, aging or the staging of AD.

Methods

We analyzed consecutive patients with AD who visited our outpatient department between January 2001 and March 2003. The diagnosis of AD was made by a neurologist based on their clinical history, DSM-IV criteria and NINCDS/ADRDA criteria [9]. Patients with a history of head injury were excluded from the study. Patients were divided into two groups, i.e., AD patients without CVD and AD patients with CVD. If a patient with AD also has a history of CVD or if MRI scan demonstrates any infarction, the patient is excluded from the group AD without CVD. If MRI demonstrates silent infarction or macrohemorrhage, but the lesions are not related to dementia in a patient with AD, the patient is classified as AD with CVD. White matter lesion demonstrated by MRI scanning was not considered for the classification of AD with CVD. The age-matched controls were recruited from the subjects who visited our outpatient clinic and had no abnormalities proved both by neurological examinations and MRI findings.

MRI scans were obtained with a 1.5-tesla system (Gyroscan Intera, Philips). Twenty-one contiguous axial or coronal 5-mm-thick slices (interslice gaps: 1 mm) were attained with the following T_2*-weighted pulse sequences: repetition time 667 ms; echo time 23 ms; field of view 230 × 230 mm; matrix 256 × 256. Conventional MRI series, including T_1/T_2-weighted and FLAIR images, were also obtained.

Two raters (one neurologist and one neuroradiologist) jointly analyzed the MR images on a consensus basis. Focal areas of signal loss in the C/CS on the T_2*-weighted images were defined as microbleeds. In our study, areas of symmetric hypointensity in the globus pallidus and flow voids of cortical arteries were excluded. Vascular flow void artifacts were excluded by tracing areas of local signal loss on adjacent slices. Cerebral white matter lesions were classified as (0) normal, (1) punctuate, (2) early confluent and (3) confluent according to the criteria by Fazekas et al. [10]. We considered groups 2 and 3 to involve confluent white matter lesions.

From the clinical records, we retrospectively obtained clinical information on age, gender, systolic and diastolic blood pressure, Mini Mental State Examination (MMSE) score and risk factors for stroke (hypertension, hyperlipidemia, diabetes mellitus and use of antiplatelet agents). We then compared these clinical backgrounds between the microbleed-positive group and its negative counterpart in each of the two groups: AD with CVD and AD without CVD. Statistical analysis was performed with StatView (Abacus Concepts, Calif., USA). The Mann-Whitney U test or χ^2 test compared the clinical backgrounds in each group. A value of $p < 0.05$ was considered to be significant.

Results

Forty-two AD patients without CVD (12 male and 30 female), 8 AD patients with CVD (5 male and 3 female) and 26 aged-matched controls were enrolled in this study. In the AD without CVD group, probable AD was diagnosed in 32 and possible AD in 10 patients based on NINCDS/ADRDA criteria. In the AD with CVD group, there were no patients with a history of hemiparesis, sen-

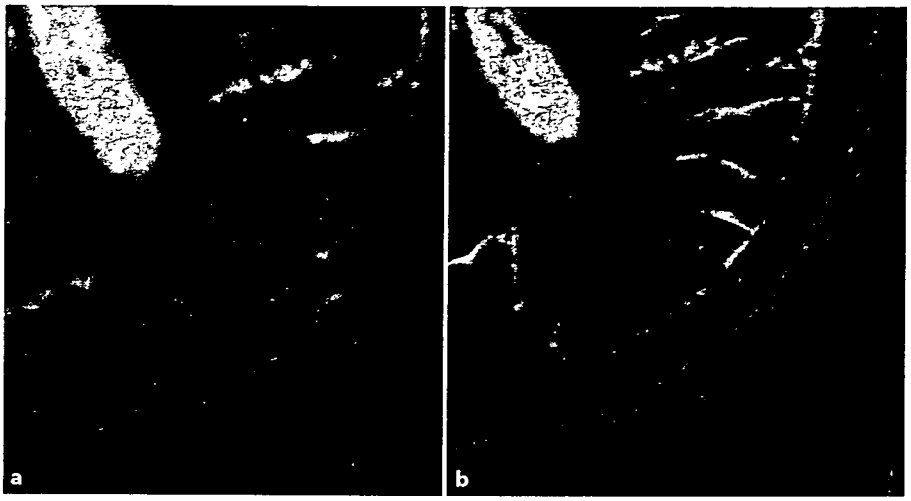

Fig. 1. a The arrow heads show two microbleeds in T_2* at the left parietotemporal lobe in an AD patient. **b** These microbleeds were not detected in conventional T_2-weighted imaging.

Table 1.

	AD without CVD		AD with CVD		Control
	MB(+)	MB(−)	MB(+)	MB(−)	
Number	7	35	1	7	26
Age, years	74.3 ± 7.7[a, b]	74.5 ± 8.2[b]	75[a, b]	74.9 ± 5.2[b]	71.2 ± 6.4
Male, %	28.67[c, d]	28.6[d]	100[c, d]	57.1[d]	38.5
Hypertension, %	42.9[c, d]	45.7[d]	100[c, d]	57.1[d]	38.5
Blood pressure, mm Hg	143/82[a, b]	132/75[b]	144/83[a, b]	146/79[b]	139/78
Hyperlipidemia, %	57.1[c, d]	28.6[d]	0[c, d]	14.3[d]	30.8
Diabetes mellitus, %	28.6[c, d]	14.3[d]	100[c, d]	14.3[d]	11.5
White matter lesions, %	57.1*[c]	22.0[d]	0[c,d]	25.0[d]	15.4
Antiplatelet agents, %	0[c]	11.4	100[c]	14.3	
MMSE score	21.3 ± 3.8[a]	19.3 ± 5.4	21[a]	18.0 ± 4.6	

Risk factors for microbleeds (MB). * $p = 0.023$ by χ^2 test [MB(+) compared with MB(−)].
[a] Not significant by Mann-Whitney U test [MB(+) compared with MB(−), $p < 0.05$].
[b] Not significant by Mann-Whitney U test [MB(+) compared with control, $p < 0.05$].
[c] Not significant by χ^2 test [MB(+) compared with MB(−), $p < 0.05$].
[d] Not significant by χ^2 test [MB(+) compared with control, $p < 0.05$].

sory loss, visual defect or ataxia. Only 1 patient complained of dizziness and MRI demonstrated infarction at the right putamen. MRI demonstrated silent infarction in 7 other patients. These infarctions could not explain cognitive decline. Therefore, all these AD patients with CVD satisfied the criteria of NINCDS/ADRDA. No significant difference between each group was observed regarding age, gender, blood pressure or history of hypertension, hyperlipidemia, diabetes mellitus or the grading of white matter lesions, although the number of AD patients with CVD was small.

T_2*-weighted imaging revealed microbleeds in 7 AD patients without CVD (16.7%) and 1 AD patient with CVD (12.5%), but no microbleeds were found in the control subjects ($p < 0.05$; χ^2 test, compared with control subjects). All hemorrhages detected were 5 mm or less in diameter. Multiple microbleeds were detected in 3 patients and single microbleeds in 5 patients. They were located either in the cortex or subcortical white matter, evading basal ganglia, cerebellum or the pontine base. Conventional T_2-weighted images did not demonstrate any of these lesions (fig. 1).

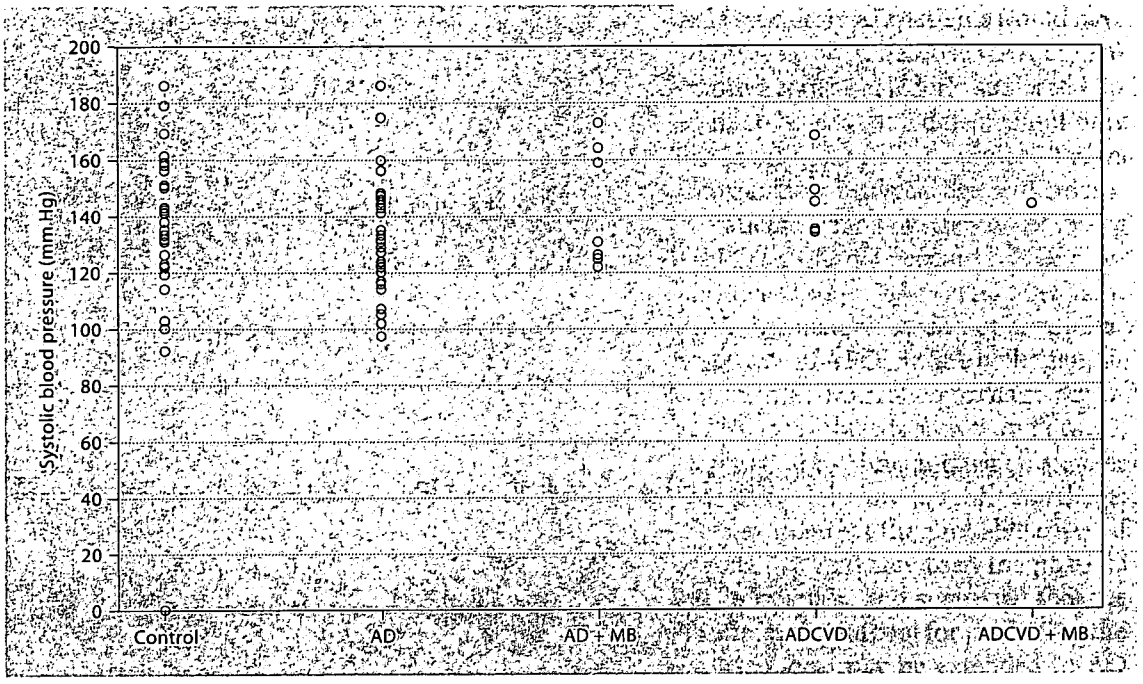

Fig. 2. Average systolic blood pressure during 1 year prior to MRI scanning in each case in the control and AD groups. AD cases were divided into 4 groups: AD with microbleeds (AD + MB), AD without microbleeds (AD), AD with CVD and microbleeds (ADCVD + MB), and AD with CVD but not microbleeds (ADCVD).

Unexpectedly, no significant difference was observed with regard to age, gender, MMSE scores or history of hypertension, hyperlipidemia, diabetes mellitus or the use of antiplatelet agents between the microbleed-positive and -negative group of AD with or without CVD. Neither systolic nor diastolic blood pressure in AD patients differed from those in the control. The averages for systolic and diastolic blood pressure in the microbleed-positive group were 9.0 and 6.7 mm Hg higher than those in the microbleed-negative group, respectively, but these differences were not significant (table 1). Figure 2 shows the systolic blood pressure of each AD case in the microbleed-positive and -negative group. Systolic blood pressure was above 159 mm Hg in 3 cases of AD with microbleeds diagnosed as having hypertension and treated with depressor, but systolic hypertension was below 131 mm Hg in 4 cases of AD with microbleeds. The frequency of confluent white matter lesions was significantly higher in the microbleed-positive group than in the microbleed-negative group ($p = 0.023$; table 1).

During the follow-up period, 1 AD patient with microbleeds, an 81-year-old female, developed a clinically overt brain hemorrhage. She visited our hospital for forgetfulness, but her MMSE score was full at the first visit, however, her cognitive function gradually worsened over the next year. She was then diagnosed as having dementia based on DSM-IV and her T_2*-weighted images revealed microbleeds in the left occipital lobe. One year after diagnosis, she suddenly lost her consciousness and was transferred to our hospital. Brain CT on admission revealed a large lobar hemorrhage in the left temporo-occipital cortex (fig. 3). The site of this lobar hemorrhage corresponded to the site of the microbleeds in the first T_2*-weighted images. She received conservative therapy, but both physical and cognitive function became severely disturbed. She was transferred to another hospital to receive care as a bedridden patient.

Discussion

After intracranial hemorrhage, hemosiderin remains stored in macrophages, and because of its magnetic properties, this leads to focal dephasing of the MRI signal causing hemosiderin-containing areas to appear hypointense on T_2-weighted spin-echo sequences. This effect can be further enhanced by using imaging techniques with high sensitivity to magnetic susceptibility, such as the gradient-

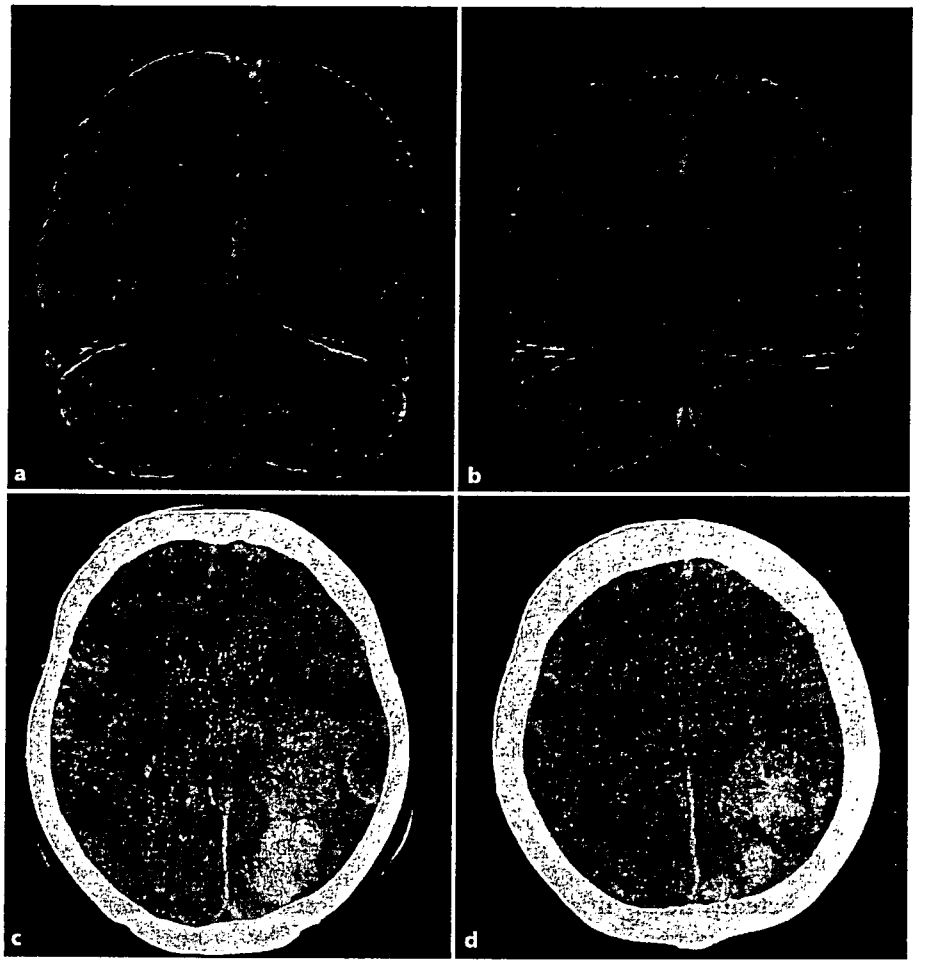

Fig. 3. a, b T_2* images showing microbleeds in the left occipital lobe of an 81-year-old female diagnosed with AD. **c, d** The patient was transferred to our hospital for loss of consciousness 2 years after her first visit. The CT scan revealed a lobar hemorrhage in the left temporo-parieto-occipital cortex.

echo sequences [7, 11]. Fazekas et al. [4] have shown in their series of 11 autopsied brains from people who died from cerebral hemorrhage that T_2*-weighted images were extremely sensitive in depicting microbleeds. They carefully ruled out the possibilities of intracerebral calcification or vascular malformation, which are other possible mechanisms of T_2 shortening.

A significant proportion of spontaneous lobar hemorrhage in the elderly is due to CAA. CAA can cause microbleeds as well as large lobar hemorrhages [7]. Microbleeds in CAA are in general situated in C/CS regions. Microbleeds of hypertensive patients or lacunar stroke patients, on the other hand, are noted at the basal ganglia, thalamus and cerebellum [8, 12]. Therefore, these two microbleeds can be roughly differentiated by their locations [7, 13].

There are several clinically significant issues in detecting microbleeds using T_2*-weighted images. For instance, hereditary CAA patients are known to reveal microbleeds in C/CS regions before symptomatic lobar hemorrhage [14]. Microbleeds were detected in almost all patients with lobar macrohemorrhages, and the microbleeds were nearly 2.5-fold more common than macrohemorrhage in CAA patients [15]. In addition, Greenberg et al. [15] reported that 47% of probable CAA patients showed new hemorrhages during the next 17-month follow-up. Therefore, it is conceivable that microbleeds detected by T_2*-weighted images will help not only in making presymptomatic diagnoses, but also in monitoring the progression of CAA. The detection rate of microbleeds in our AD patients without CVD was 16.7%, whereas there were no microbleeds in any of the aged-matched controls. The rate of microbleeds for the control subjects in our population is thought to reside within the range reported by Kinoshita et al. [8] who found silent microbleeds in the C/CS area in only 1 patient (1.5%) of 66 healthy Japanese elderly subjects, but was somewhat lower than the results from the Framingham study in which there was a 4.7% incidence of microbleeds for healthy American elderly people [13].

The rate of microbleeds in our AD patients was much higher than any of these control data.

We speculated, prior to the study, that hypertension or the use of antiplatelet or anticoagulant agents would have adverse effects on microbleeds. This retrospective study has a limitation in discussing the risk factors of microbleeds in AD; however, these factors did not have a significant relationship with the presence of microbleeds for the AD without CVD group. Particularly, systolic blood pressure was not related to hemorrhage in more than half of the AD cases. Furthermore, hyperlipidemia and diabetes mellitus also had no significant correlation with microbleeds. These data may also support the hypothesis that microbleeds detected in AD patients are not related to CVD, but are more so to CAA. In the AD with CVD group, microbleeds were detected in only 1 patient; therefore, more samples need to be accumulated in order to discuss the relation between microbleeds and CVD or hemorrhagic risk factors.

In our study, confluent white matter lesions were more frequent in the microbleed-positive group than in the microbleed-negative group or the normal controls. Cerebral white matter lesions are known to be related to age, hypertension, diabetes mellitus, hyperlipidemia and heart disease [4, 16], but these factors were not related to microbleeds in our study. White matter lesions are also frequently accompanied by hereditary or sporadic CAA due to amyloid deposition on meningeal and subcortical small arteries and the subsequent obstruction of these arteries [17, 18]. The relation between microbleeds and white matter lesions in our AD patients may further indicate that the microbleeds in AD are related to CAA.

We also speculated that elderly AD patients would have more microbleeds in the neocortex because CAA in general is known to be more frequent in elderly subjects. Unexpectedly, however, microbleeds were detected not only in elderly AD but also in mild AD patients under 70 years of age. The lack of correlation between age and microbleeds is related to patients with younger onset being more likely to have APOE epsilon 4 because APOE epsilon 4 predisposes to a greater amyloid burden in the brain and cerebral vessels. Unfortunately, we do not have sufficient data to discuss the role of APOE in the pathogenesis of microbleeds in this study. Macrohemorrhage developed in an AD case with microbleeds shown in figure 3, suggesting the importance of T_2* imaging which enables the detection of AD with CAA before lobar hemorrhage. Greenberg et al. [15] reported that the probable cases of CAA have a higher bleeding tendency than the possible cases of CAA during their course and suggested that the detection of microbleeds is a useful tool for predicting the progression of CAA. This idea may be extended to AD patients. The detection of CAA in AD may give us important information about the amyloid burden in the vessels and neurons in the brain. In the near future, amyloid imaging will also help our understanding of the amyloid deposition in vivo.

In summary, we have shown in this study that T_2* images can detect microbleeds in the C/CS area, which are more frequent in AD patients than normal controls. Microbleeds in AD, not being related to common hemorrhagic risk factors, but significantly related to white matter pathologies, are more likely caused by CAA than CVD.

References

1 Kalaria RN: Cerebrovascular degeneration is related to amyloid-beta protein deposition in Alzheimer's disease. Ann NY Acad Sci 1997; 826:263–271.

2 Olichney JM, Hansen LA, Hofstetter CR, Grundman M, Katzman R, Thal LJ: Cerebral infarction in Alzheimer's disease is associated with severe amyloid angiopathy and hypertension. Arch Neurol 1995;52:702–708.

3 Nakata Y, Shiga K, Yoshikawa K, et al: Subclinical brain hemorrhages in Alzheimer's disease: evaluation by magnetic resonance T_2*-weighted images. Ann NY Acad Sci 2002;977: 169–172.

4 Fazekas F, Kleinert R, Roob G, et al: Histopathologic analysis of foci of signal loss on gradient-echo T_2*-weighted MR images in patients with spontaneous intracerebral hemorrhage: evidence of microangiopathy-related microbleeds. AJNR Am J Neuroradiol 1999; 20:637–642.

5 Ellis RJ, Olichney JM, Thal LJ, et al: Cerebral amyloid angiopathy in the brains of patients with Alzheimer's disease: the CERAD experience. 15. Neurology 1996;46:1592–1596.

6 Hanyu H, Tanaka Y, Shimizu S, Takasaki M, Abe K: Cerebral microbleeds in Alzheimer's disease. J Neurol 2003;250:1496–1497.

7 Greenberg SM, Finklestein SP, Schaefer PW: Petechial hemorrhages accompanying lobar hemorrhage: detection by gradient-echo MRI. Neurology 1996;46:1751–1754.

8 Kinoshita T, Okudera T, Tamura H, Ogawa T, Hatazawa J: Assessment of lacunar hemorrhage associated with hypertensive stroke by echo-planar gradient-echo T_2*-weighted MRI. Stroke 2000;31:1646–1650.

9 McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM: Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939–944.

10 Fazekas F, Niederkorn K, Schmidt R, et al: White matter signal abnormalities in normal individuals: correlation with carotid ultrasonography, cerebral blood flow measurements, and cerebrovascular risk factors. Stroke 1988; 19:1285–1288.

11 Atlas S, Mark A, Grossman R, Gomori J: Intracranial hemorrhage: gradient-echo MR imaging at 1.5 T. Comparison with spin-echo imaging and clinical applications. Radiology 1988;168:803–807.
12 Fan YH, Mok VC, Lam WW, Hui AC, Wong KS: Cerebral microbleeds and white matter changes in patients hospitalized with lacunar infarcts. J Neurol 2004;251:537–541.
13 Jeerakathil T, Wolf PA, Beiser A, et al: Cerebral microbleeds: prevalence and associations with cardiovascular risk factors in the Framingham Study. Stroke 2004;35:1831–1835.
14 van den Boom R, Bornebroek M, Behloul F, van den Berg-Huysmans AA, Haan J, van Buchem MA: Microbleeds in hereditary cerebral hemorrhage with amyloidosis-Dutch type. Neurology 2005;64:1288–1289.
15 Greenberg SM, Eng JA, Ning M, Smith EE, Rosand J: Hemorrhage burden predicts recurrent intracerebral hemorrhage after lobar hemorrhage. Stroke 2004;35:1415–1420.
16 de Leeuw FE, de Groot JC, Oudkerk M, et al: Hypertension and cerebral white matter lesions in a prospective cohort study. Brain 2002;125:765–772.
17 Smith EE, Gurol ME, Eng JA, et al: White matter lesions, cognition, and recurrent hemorrhage in lobar intracerebral hemorrhage. Neurology 2004;63:1606–1612.
18 Greenberg SM, Gurol ME, Rosand J, Smith EE: Amyloid angiopathy-related vascular cognitive impairment. Stroke 2004;35:2616–2619.

Geriat. Med. 45(1) : 13~17, 2007

Geriatric Medicine（老年医学） 1月号 Vol. 45 No. 1 2007

特集：老年医学の展望

老化および老年病の疫学的研究

下方 浩史

株式会社 ライフ・サイエンス

Geriat. Med. 45(1)：13～17, 2007

特集 老年医学の展望

I

老化および老年病の疫学的研究

下方　浩史*

KEY WORD

老化
老年病
疫学
ケノム研究
長期縦断疫学研究

POINT

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

0387-1088/07/¥500/論文/JCLS

はじめに

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

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

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

老化の縦断的研究

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

*しもかた　ひろし：国立長寿医療センター研究所疫学研究部

表1　コホート研究と老化の縦断研究の比較

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

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

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

縦断的方法がなぜ必要か

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

表2　国内外の代表的な老化の縦断的研究[6]

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

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

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

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

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

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

縦断疫学研究の新たな課題

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

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

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

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

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

老化・老年病の分子疫学と縦断研究

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

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

国立長寿医療センター長期縦断疫学研究

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

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