□ ORIGINAL ARTICLE □

Lifestyle Changes and Oxidative Stress in a High-incidence Area of Amyotrophic Lateral Sclerosis in the Southwestern Kii Peninsula, Japan

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Abstract

Objective Lifestyle changes may play an important role in the incidence reduction and delay of onset age of amyotrophic lateral sclerosis (ALS) in the Koza/Kozagawa/Kushimoto (K) area. The aim of this study was to evaluate recent lifestyle changes in the K area and to investigate the relationships between lifestyle and oxidative stress among the residents.

Methods We conducted a medical checkup for elderly residents in the K area and the control area and evaluated the urinary 8-OHdG levels, cognitive function test scores and metal contents in serum and scalp hair, coupled with a lifestyle questionnaire survey between 2010 and 2015.

Results Recent lifestyle changes among the K residents, including a decrease in the Japanese pickle consumption, increase in fresh vegetable consumption and decrease in farm work, were evaluated in this study. Low consumption of Japanese pickles, high consumption of fresh vegetables, rare farm work and low levels of 8-OHdG/creatinine were all associated with high scores in the cognitive function tests. Frequent farm work and consumption of Japanese pickles was associated with high contents of transition metals, such as Mn, Al and V, in the scalp hair.

Conclusion These lifestyle changes among residents in the K area may be associated with their oxidative stress.

Key words: Kii-ALS, lifestyle change, oxidative stress, Mn, Zn

Introduction

The Koza/Kozagawa/Kushimoto (K) area in the Kii Peninsula of Japan exhibited a 10-fold higher incidence of ALS than other parts of the world in the 1950s and 1960s (1-3). The incidence of ALS in the K area, as well as in the Hohara area of the eastern part of the Kii Peninsula and in Guam, other high-incidence areas for the ALS/parkinsonism-dementia complex (ALS/PDC), have gradually decreased over the years (4-7). The age of disease onset has also increased (8). Westernization of the lifestyle and environmental factors have been suggested to play a role in the incidence decrease and delay of onset in this disease (5, 6, 8).

Environmental research has shown that the soil in the K area contained high levels of Mn and Al in the past (3, 9), and that the drinking water in the K area (sourced from the Kozagawa River) contained markedly low levels of Ca, Mg and Zn (3, 9, 10). We also found that the patients with ALS in the K area (K-ALS) and non-patient residents (K resi-

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dents) had lower serum Ca and Zn levels and higher levels of the oxidative stress markers 8-hydroxy-2'-deoxyguanosine (8-OHdG, an oxidative stress marker of DNA) and Cu/Zn than control subjects from other areas (10). The Mn and V levels in the scalp hair of patients with K-ALS and some K residents were also higher than those of the control subjects (11). The water source and the soil in the K area are the same as in the 1950s and 1960s, except for on a small island of the K area where the drinking water source was changed to the Kozagawa River in 1975, resulting in a recent increase in the incidence of ALS (12). The absorption of transition metals has been found to be enhanced under conditions of low Ca and/or Zn (13), and the accumulation of transition metals increases the oxidative stress in neurons (14).

Given these factors, we hypothesized that lifestyle changes might exert protective effects against oxidative stress and play an important role in the reduction of the incidence and delay of ALS onset age in the K area. However, lifestyle changes among the K residents and the effects of these changes on the oxidative stress have not been clarified.

Objective

The aim of this study was to clarify recent lifestyle changes in the K area and to investigate the relationships between lifestyle and oxidative stress markers among the K residents.

Materials and Methods

Between 2010 and 2015, we conducted a medical checkup for elderly residents (>65 years old) 4 times in the K area and once in each control area (H and O areas in the northern part of the Kii peninsula and S area in the eastern part of the Kii peninsula). Residents who voluntarily participated in the medical checkup were requested to answer the lifestyle questionnaire, including the consumption frequency of various foods and drinking water and their work habits. The questionnaire consisted of 63 dichotomous questions, such as "frequently"-or-"rarely" questions, and each question was to be answered both "at present" and "in the 1960s" by memory. In the questionnaire, "frequently" meant "every day or every other day" and "rarely" meant "less than once a week". We explained to the residents that the "in the 1960s" meant the time when they had been around 20-30 years old.

The medical checkup included physical measurements, a neurological examination, blood (including a measurement of serum Ca, Mg, Zn, Cu, albumin, aspartate aminotransferase (AST), and alkaline phosphatase (ALP)) and urine biochemistry (including a routine dipstick test and measurement of 8-OHdG), and tests of the cognitive function, including a Mini-Mental State Examination (MMSE), Hasegawa Dementia Scale-revised (HDS-R), Frontal assessment battery (FAB) and a scent identification card test (Open Essence, Wako, Japan). The scent identification card test

"Open Essence" was reported to be useful as a screening test of the olfactory function (15). Deterioration of the olfactory function is an early sign of cognitive impairment (16). Scalp hair samples from the K residents were collected during the research period between 2010 and 2011, and the metal levels were determined by a neutron activation analysis (NAA) at Kyoto University Research Reactor (KUR) between 2011 and 2012. Details regarding conducting measurements via a NAA at KUR have been previously reported (11).

Based on the questionnaire, we examined the lifestyle characteristics of the K residents compared with the control areas, both in the 1960s and "at present". Among the K residents, we analyzed the relationships between the lifestyle change and the medical checkup data, including the 8-OHdG/creatinine (8-OHdG/c) and transition metal contents in the scalp hair, with special reference to the oxidative stress.

Statistical analysis

The data were expressed as the mean ± standard deviation (SD) or standard error (SE). A p value of <0.05 was considered statistically significant. The software program SPSS version 11 (SPSS, Chicago, IL, USA) was used for all statistical analyses.

Ethics statement

This study was approved by the Institutional Ethics Committee of the Kansai University of Health Sciences (10-03, 12-16 and 14-07). All participants provided informed consent and voluntarily agreed to participate in this study.

Results

Lifestyle changes in the K area

A total of 160 K residents without duplication and 62 control area residents participated in this study and voluntarily received a medical checkup, and 93 of the K residents and 62 control residents answered the questionnaire during the research period. In the K area, the cumulative participant during the 4 medical checkups was 352, and 160 of these 352 persons were unique individuals, so the first-time data of these 160 K residents were used to prevent overlap. We analyzed the relationships between lifestyle and the medical checkup data of the 93 K residents who answered the questionnaire in this study (Table).

The proportion of K residents who answered that they frequently ate Japanese pickles (p<0.05) and dried fish (p<0.05) at 20-30 years of age (in the 1960s) was significantly higher than that of the controls at the time. The proportion of K residents who answered that they frequently ate eggs and egg dishes (p<0.05), milk and dairy products (10 points) and meat dishes (12 points) in the 1960s was lower than that of the controls (Fig. 1a). On comparing the responses between the 1960s and present, the percentage of K resi-

Table.   Participants in the Study between 2010 and 2015.

	K area	Control
Participants	160*	62
Respondent to the questionnaire	93	62
Age (mean±S.D.)	76.9±7.7	73.8±6.5
Medical checkup analyzed in this study		
Measurement of metals	Questionnaire respondents** / total***	
Metals in the serum	93#/160	
Metals in the scalp hair	55/132	
Cognitive function tests		
HDS-R	53/75	
MMSE	53/75	
FAB	53/74	
Scent identification card test	92/93	
Measurement of 8-OHdG/creatinine	29/93	

The population of the K area in 2010 was 22044, and 8333 people were ≥65-years-old according to the Wakayama Prefectural 2010 census.

*: The cumulative number who participated in 4 medical checkups was 352, and 160 of these were unique individuals. The first-time data of these 160 K residents were analyzed.

**: The sample number from the questionnaire respondents is shown.

***: The total sample number from the participants without duplication is shown.

#: The levels of Ca and Zn from 28 of 93 serum samples were excluded from the analysis due to postprandial collection.

dents who answered that they frequently eat eggs and egg dishes, milk and dairy, fresh vegetables and bread increased 23.8%, 29.2%, 20.6% and 40.2%, respectively. In contrast, the percentage of K residents who answered that they frequently ate rice, Japanese pickles and miso soup at present had decreased 25.2%, 16.7% and 34.8%, respectively, compared with the 1960s (Fig. 1b). Although the percentage of K residents who answered that they frequently ate bread, miso soup and seaweed were different from those of the controls (p<0.05, respectively), the eating pattern of the K residents at present was fairly well-balanced, similar to that of the controls (Fig. 1c).

Regarding the source of their drinking water, the percentage of tap water drinkers in the K area at present was 87%, which was 55 points higher than in the 1960s. The percentage of well water users decreased from 44% in the 1960s to 1.4% at present, and that of spring water users decreased from 35% in the 1960s to 8.5% at present. Purchasing fish at supermarkets at present had increased 37 points (55%) compared with in the 1960s (18%). Similarly, purchasing vegetables (from 25% in the 1960s to 54% at present, respectively), fruit (from 21% to 69%) and meat (from 35% to 69%) were also increased at present compared with in the 1960s. The percentages of residents who answered that they frequently engaged in heavy physical labor (from 56% to 10%), fishing (from 32% to 7%), forestry (from 7% to 0%) and paddy agriculture (from 42% to 3%) in the K area was

markedly lower at present than they had been in the 1960s. Meanwhile, the percentage of the K residents who answered that they frequently do farm work was 39% at present and 51% in the 1960s.

To evaluate the effects of lifestyle changes on the medical checkup data of the K residents, the questionnaire answers were divided into four groups based on lifestyle changes: rarely in the 1960s and rarely at present (r→r group), rarely in the 1960s and frequently at present (r→f group), frequently in 1960s and frequently at present (f→f group) and frequently in the 1960s and rarely at present (f→r group). Of note, concerning the consumption of Japanese pickles and fresh vegetables, the cognitive function test scores were significantly different among the four groups (p<0.05, respectively, ANOVA), and concerning the frequency of farm work, the scalp hair Al contents were significantly different among the four groups (p<0.05, ANOVA).

Relationships between the changes in the consumption of Japanese pickles and cognitive function test scores, 8-OHdG/c levels and hair metal contents among K residents

The MMSE scores were significantly different among the four groups of Japanese pickle consumption. The MMSE scores of the K residents who answered that they rarely ate Japanese pickles both in the 1960s and at present (r→r group) were significantly higher than those who frequently ate them in both the 1960s and at present (f→f group, p<0.05) and those who frequently ate them in the 1960s but rarely at present (f→r group, p<0.05), and tended to be higher than those of the r→f group. In contrast, the MMSE scores of the f→f group tended to be lower than those of the f→r group (Fig. 2a). The FAB scores were significantly different among the four groups, and the scores of the r→r group were higher than those of the f→f group (p<0.05, Fig. 2b). The scent identification card test scores (scent scores) were not significantly different among the four groups (Fig. 2c).

The urinary levels of 8-OHdG/c were measured in the K residents who did not have smoking habits, overt kidney disease or liver disease in the medical checkup. The 8-OHdG/c levels of the r→r group tended to be lower than those of the r→f and f→f groups, but they were not significantly different among the four groups (Fig. 2d). The hair Mn and V contents were significantly different among the four groups. The hair Mn contents of the r→r group were lower than those of the f→f group (Fig. 2e). The hair V contents of the r→r group were lower than those of the f→f group (p<0.05, Fig. 2f).

Relationships between the changes in the consumption of fresh vegetables and cognitive test scores, 8-OHdG/c levels and hair Mn and V contents among K residents

The MMSE scores were not significantly different among the four groups of consumption of fresh vegetables. How-
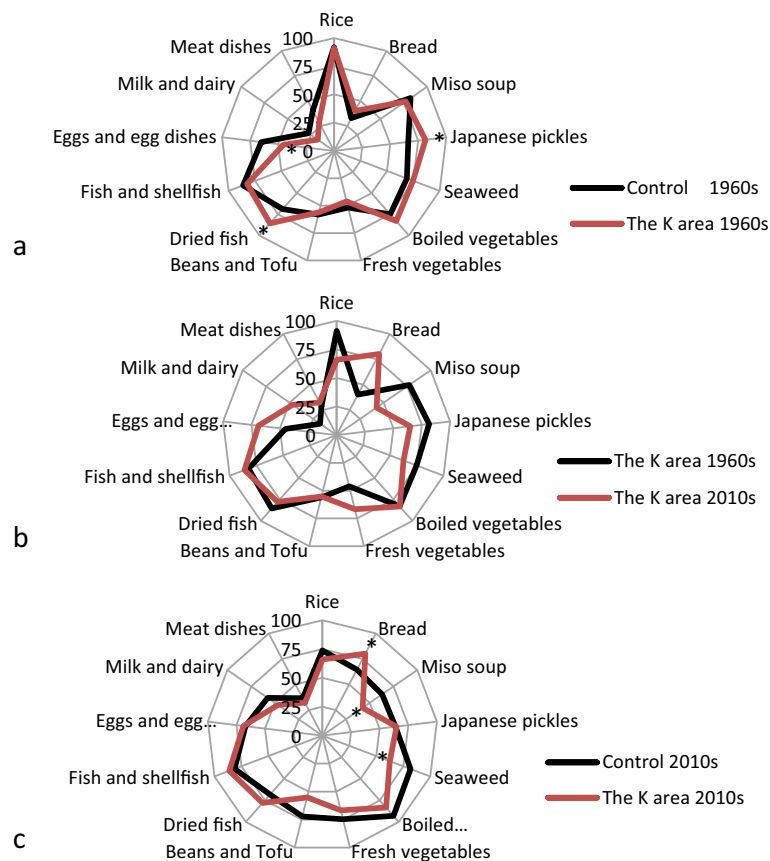
Figure 1.   Laser charts of the lifestyle changes in the K area between the 1960s and present. The percentages of K residents who answered that they frequently ate selected food items were compared with those of the controls in the 1960s (a). The percentages of K residents who answered that they frequently ate selected food items were compared between the 1960s and present (in the 2010s) (b). The percentages of K residents who answered that they frequently ate selected food items at present (in the 2010s) were compared with those of the controls at present (in the 2010s) (c). *: p<0.05

ever, on comparing the r→r group and r→f group, the MMSE scores of the r→r group were significantly lower than those of the r→f group (p<0.05, Fig. 3a). The FAB scores were not significantly different among the four groups. On comparing the r→r group *and* r→f group, the FAB scores tented to be lower in the r→r group than in the r→f group (p=0.056) (Fig. 3b). The scent scores were significantly different among the four groups, and the scores of the r→r group were lower than those of the f→f group. Furthermore, on comparing the f→f group and the f→r group, the scent scores of the f→f group tended to be higher than those of the f→r group (p=0.058) (Fig. 3c). The 8-OHdG/c levels were significantly different among the four groups, and the levels of the f→f group were significantly lower than those of the f→r group (p<0.05) (Fig. 3d). The 8-OHdG/c levels of the f→r group were significantly higher than those of the r→r group. The hair Mn and V contents were not significantly different among the four groups (Fig. 3e and f).

Relationships between the frequency of farm work and cognitive function test scores, 8-OHdG/c levels and hair Mn and Al contents among K residents

The MMSE scores were not significantly different among the four groups of farm work frequency. On comparing between the f→f group and the r→r group, the MMSE scores of the f→f group tended to be lower than those of the r→r group (p=0.07, Fig. 4a). The FAB scores and the scent scores were not significantly different among the four groups (Fig. 4b and c). On comparing the r→r group and the f→f group, the scent scores of the r→r group were higher than those of the f→f group (p=0.02, Fig. 4c). There were no significant differences in the 8-OHdG/c levels among the four groups. On comparing the r→f group and the f→r group, the 8-OHdG/c levels of the r→f group tended to be lower than those of the f→r group (p=0.06, Fig. 4d). The hair Mn contents were not significantly different among the four groups. On comparing the r→r group and the f→f group, the hair Mn contents of the r→r group were lower than those of the f→f group (p=0.02) and tended to be lower than those of the r→f group (p=0.04, Fig. 4e). The hair Al contents were significantly different among the

r→r: rarely in the 1960s and rarely at present
r→f: rarely in the 1960s and frequently at present
f→f: frequently in the 1960s and frequently at present
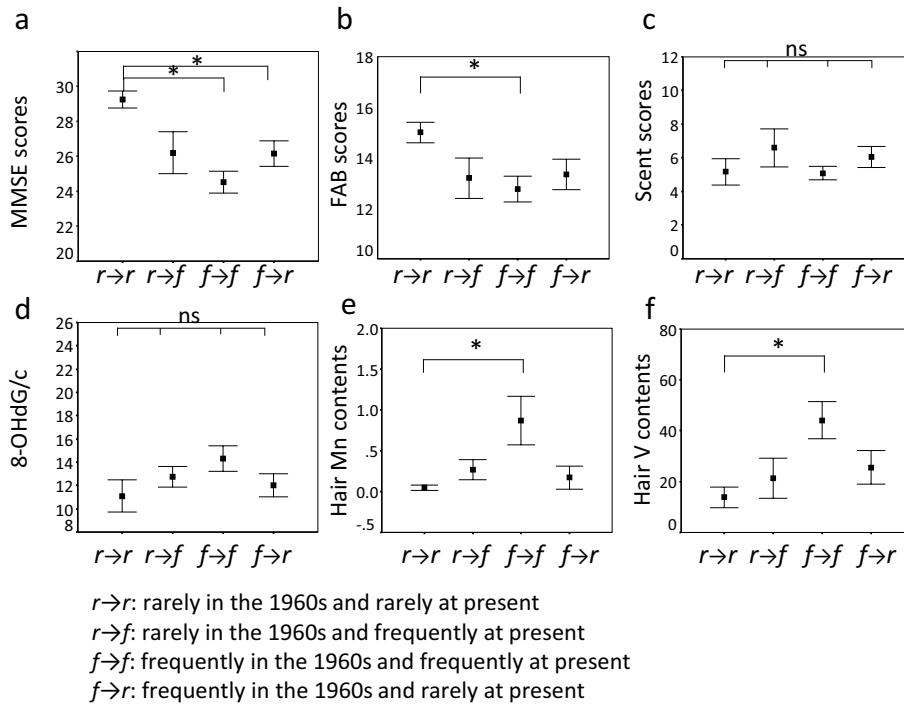f→r: frequently in the 1960s and rarely at present

Figure 2. **The relationships between the changes in the consumption of Japanese pickles and cognitive function test scores, 8-OHdG/c levels and hair Mn and V contents. The labels of the x bars from Figs. 2 to 4 are as follows: *r→r*: rarely in the 1960s and rarely at present, *r→f*: rarely in the 1960s and frequently at present, *f→f*: frequently in the 1960s and frequently at present, *f→r*: frequently in the 1960s and rarely at present. The MMSE scores of the *r→r* group were significantly higher than those of the *f→f* and the *f→r* groups (a). The FAB score of the *r→r* group were higher than those of the *f→f* group (b). The scent scores were not significantly different among the four groups (c). The 8-OHdG/c levels (ng/mg of creatinine) were not significantly different among the four groups (d). The hair Mn contents (μg/g wet weight) of the *r→r* group were lower than those of the *f→f* group (e). The hair V contents (ng/g wet weight) of the *r→r* group were significantly lower than those of the *f→f* groups (f). *: $p < 0.05$, ns: not significant. The plot and bar show the mean value and standard error, respectively.**

four groups. The hair Al contents of the *r→r* group were lower than those of the *f→f* ($p < 0.01$) and the *f→r* groups ($p < 0.05$, Fig. 4f).

Relationships between the cognitive function test scores by age and lifestyle at present among K residents

The relationship between the frequency of Japanese pickle consumption at present and the cognitive function test scores was analyzed by age. Given that the cognitive function test scores were negatively correlated with age, the scores were analyzed for the following age groups: young-old group, ≤69 years old (n=13); middle-old group (mid-old), 70-79 years old (n=37); old-old group, ≥80 years old (n=43). The K residents in the mid-old group who answered that they frequently ate Japanese pickles at present (*f* group) had lower scores in MMSE ($p < 0.05$, Fig. 5a) and FAB ($p < 0.05$, Fig. 5b) than those who rarely ate Japanese pickles at present (*r* group). The K residents who answered that they frequently ate Japanese pickles at present showed higher contents of Mn ($p < 0.05$, Fig. 5c) and V ($p < 0.05$, Fig. 5d) in the scalp hair than those who rarely ate Japanese pickles at present.

The relationship between the frequency of fresh vegetable consumption at present and the cognitive function test scores was analyzed by age. In the old-old group, the MMSE scores of the K residents who answered that they frequently ate fresh vegetables at present (*f* group) were higher than in those who rarely ate fresh vegetables at present (*r* group) ($p < 0.01$, Fig. 5e). The scent scores of the K residents in the young-old and old-old groups who answered that they frequently ate fresh vegetables at present were higher than in those who rarely ate fresh vegetables at present ($p < 0.05$ respectively, Fig. 5f). The hair Mn and V contents of the K residents who answered that they frequently ate fresh vegetables at present were not markedly different from those who rarely ate fresh vegetables at present (Fig. 5g and h).

The relationship between the frequency of farm work at present and the cognitive function test scores was analyzed by age. The K residents in the old-old group who answered that they frequently do farm work at present (*f* group) had lower MMSE scores than those who rarely do farm work at present (*r* group) ($p < 0.05$, Fig. 5i), and the residents in the mid-old group who answered that they frequently do farm work at present showed lower scent scores than those who rarely do farm work at present ($p < 0.05$, Fig. 5j). The K resi-
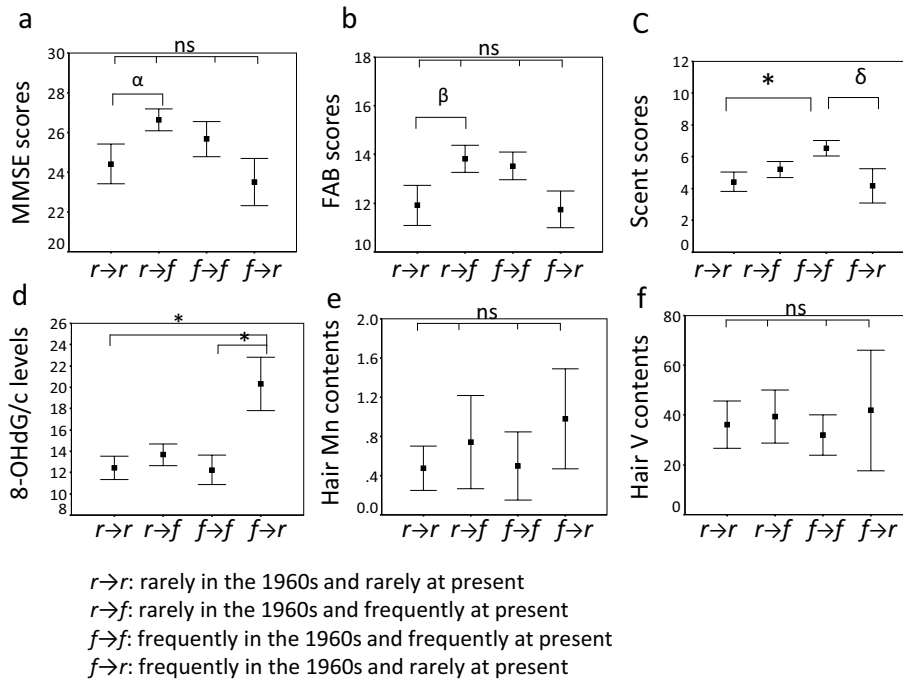
r→r: rarely in the 1960s and rarely at present
r→f: rarely in the 1960s and frequently at present
f→f: frequently in the 1960s and frequently at present
f→r: frequently in the 1960s and rarely at present

Figure 3. **The relationships between the changes in the consumption of fresh vegetables and cognitive function test scores, 8-OHdG/c levels and hair Mn and V contents. The MMSE scores were not significantly different among the four groups. However, on compareing the r→r group and the r→f group, the MMSE scores of the r→r group were lower than those of the r→f group (a). The FAB scores of the r→r group tended to be lower than those of the r→f group, on comparison between the r→r group and the r→f group (b). The scent scores of the r→r group were lower than those of the f→f group, and the scent scores of the f→f group tended to be higher than those of the f→r group (c). The 8-OHdG/c levels of the f→f group were significantly lower than those of the f→r group. The 8-OHdG/c levels of the f→r group were significantly higher than those of the r→r group (d). The hair Mn and V contents were not significantly different among the four groups (e and f). *: p<0.05, α: p=0.045, β: p=0.056, δ: p=0.058, ns: not significant. The plot and bar show the mean value and standard error, respectively.**

dents who answered that they frequently do farm work at present exhibited higher Mn (p<0.05, Fig. 5k) and Al contents (p<0.01, Fig. 5l) in their scalp hair than those who rarely do farm work at present.

Relationships between the cognitive function test scores and oxidative stress among K residents

The mean value of 8-OHdG/c in the total K residents was 11.8 ng/mg of creatinine (SD: 5.2). The values of 8-OHdG/c did not correlate with age, body mass index, blood pressure or pulse and showed mild negative correlations with serum Zn levels (r: -0.30, p<0.05), HDS-R scores (r: -0.30, p<0.05) and MMSE scores (r: -0.28, p<0.05). The K residents were categorized by the mean value of their 8-OHdG/c levels into either a high 8-OHdG/c group (≥11.8 ng/mg creatinine) or a low 8-OHdG/c group (<11.8 ng/mg creatinine). The HDS-R scores of the low 8-OHdG/c group were significantly higher than those of the high 8-OHdG/c group in the young-old group (p<0.05) and tended to be higher in the mid-old group (p=0.08, Fig. 6a). The MMSE scores of the low 8-OHdG/c group were significantly higher than those of the high 8-OHdG/c group in the mid-old group (p<0.05) and

tended to be higher than those of the high 8-OHdG/c group in the young-old group (p=0.1, Fig. 6b).

The contents of Mn (Pearson's r=0.42, p=0.053, not shown) and V (Pearson's r=0.56, p<0.05, Fig. 6c) in the scalp hair of the K residents were positively correlated with the levels of urinary 8-OHdG/c. The content of Mn in the scalp hair of the K residents was negatively correlated with the HDS-R scores (Pearson's r=-0.45, p=0.006, not shown).

Discussion and Conclusion

In this study, we found that recent lifestyle changes among the K residents were characterized by a decrease in the Japanese pickle consumption, an increase in the fresh vegetable consumption, a well-balanced diet, globalization of food acquisition and a decrease in the frequency of heavy agricultural labor. Recent trends in diet changes in the control areas obtained from our questionnaire were consistent with the literature (17, 18). Therefore, we consider that the answers from the residents about their lifestyle at present and in the 1960s, when they were 20-30 years old, reflected reasonable trends in change, although memory bias may ex-

r→r: rarely in the 1960s and rarely at present
r→f: rarely in the 1960s and frequently at present
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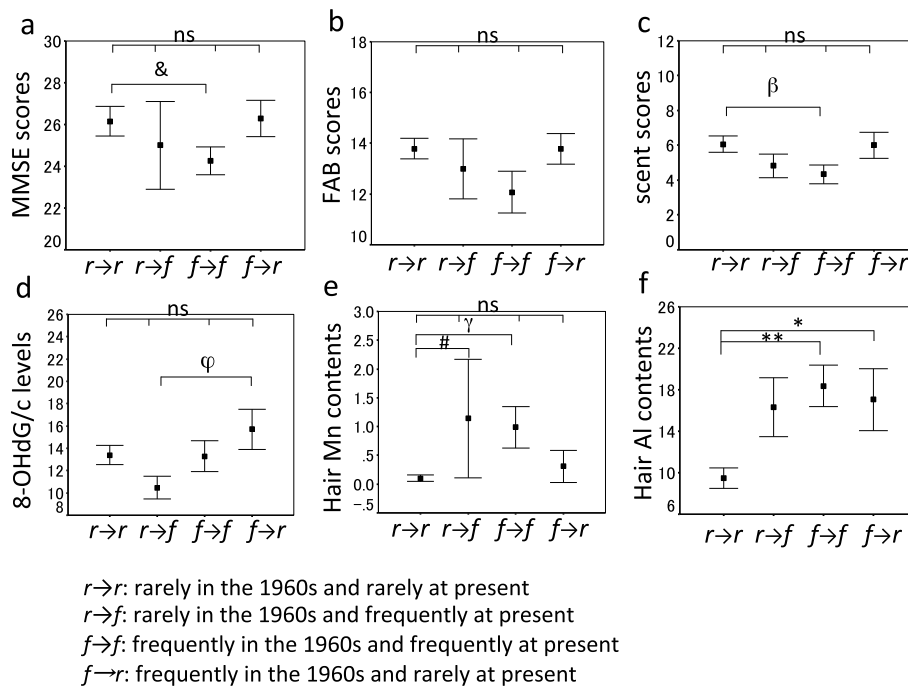f→r: frequently in the 1960s and rarely at present

Figure 4.   The relationships between the frequency of farm work and cognitive function test scores, 8-OHdG/c levels and hair Mn and Al contents. The MMSE scores were not significantly different among the four groups. On comparing the f→f group and the r→r group, the MMSE scores of the f→f group tended to be lower than those of the r→r group (a). The FAB scores were not significantly different among the four groups (b). The scent scores were not significantly different among the four groups. On comparing the r→r group and the f→f group, the scent scores of the r→r group were higher than those of the f→f group (c). The 8-OHdG/c levels were not significantly different among the four groups. On comparing the r→f group and the f→r group, the 8-OHdG/c levels of the r→f group tended to be lower than those of the f→r group (d). The hair Mn contents were not significantly different among the four groups. On comparing the r→r group and the f→f group, the hair Mn contents of the r→r group were lower than those of the f→f group and tended to be lower than those of the r→f group (e). The hair Al contents of the r→r group were lower than those of the f→f and the f→r groups (f). *: p<0.05, **: p<0.01, &: p=0.07, β: p=0.02 , φ: p=0.06, γ: p=0.02, #: p=0.04, ns: not significant. The plot and bar show the mean value and standard error, respectively.

ist.

To evaluate the relationships between recent lifestyle changes and oxidative stress, we measured the 8-OHdG/c levels, the transition metal contents in the scalp hair and the cognitive function test scores as markers of oxidative stress in this study. Increasing evidence supports the notion that the cognitive function is impaired by excessive oxidative stress (19, 20). The K residents who reduced the frequency of Japanese pickle consumption or rarely ate Japanese pickles showed higher MMSE and FAB scores and lower Mn and V contents in the scalp hair than those who frequently consumed Japanese pickles. The contents of Mn and V in the scalp hair of the K residents was positively correlated with the levels of 8-OHdG/c, and the residents with low 8-OHdG/c levels demonstrated higher scores in HDS-R and MMSE than those with high 8-OHdG/c levels. Although the causes are not clear, the decrease in the Japanese pickle consumption and the low contents of transition metals in the scalp hair may be associated with high cognitive function test scores among K residents. In the 1960s, the consump-

tion of Japanese pickles and salted dried fish with rice among K residents was significantly higher than in the controls, while the consumption of egg dishes and meat dishes was significantly lower, indicating that the diet of the K residents in the 1960s was high in carbohydrates and salt and low in fat, protein and minerals. These trends were also found in our previous study between 2004 and 2005 in the Kozagawa area, as evaluated by a self-administered food frequency questionnaire (21). A high intake of carbohydrates and low intake of fat has been associated with an increase in the odds of having ALS (22), and the diet of the K residents in the 1960s obtained by the present study was similar to the eating habits seen in patients with sporadic ALS three years prior to disease onset (22).

The K residents with an increased frequency of fresh vegetable consumption at present compared with the 1960s showed higher cognitive function test scores than those who continued rare consumption without change. The K residents with a decreased frequency of fresh vegetable consumption at present compared with the 1960s showed higher levels of
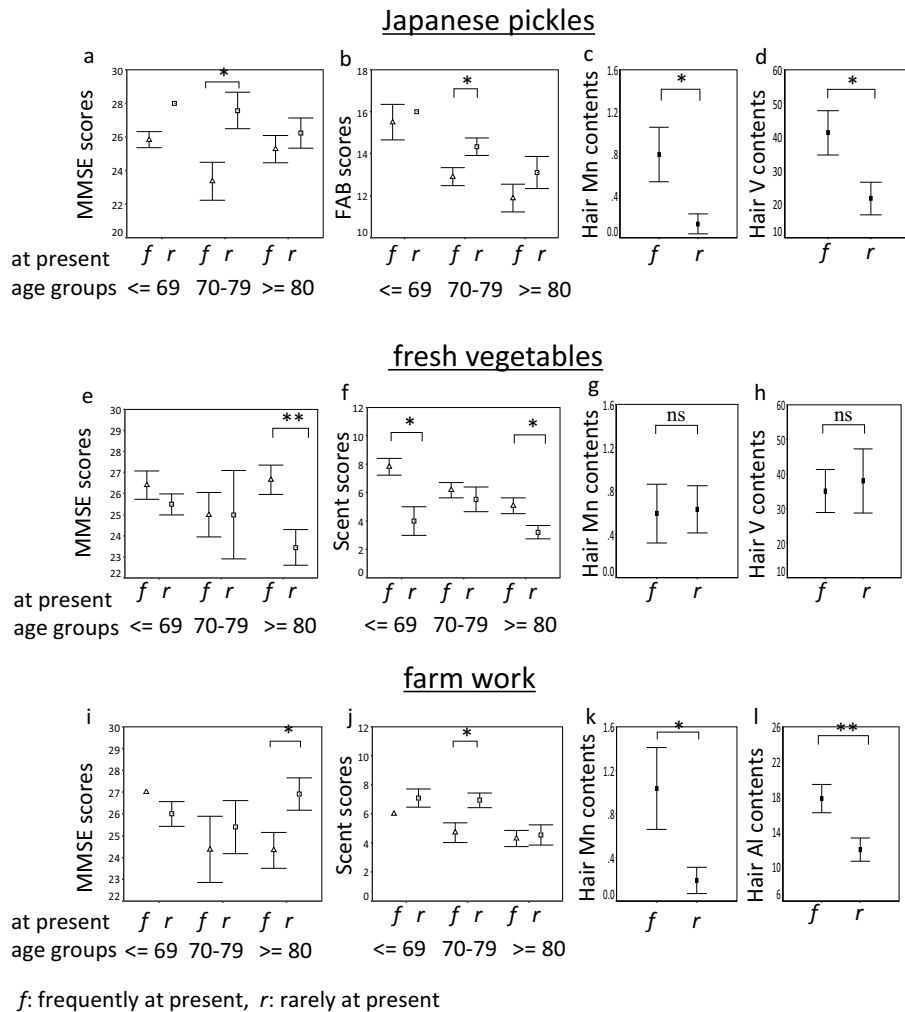
Figure 5. The relationships between the cognitive function test scores by age and lifestyle at present. For the frequency of Japanese pickle consumption, the MMSE scores of the *f* group were lower than those of the *r* group in the mid-old group (70-79 years old) (a). The FAB scores of the *f* group were lower than those of the *r* group in the mid-old group (b). The hair Mn (c) and V contents (d) of the *f* group were higher than those of the *r* group. For the frequency of fresh vegetable consumption, the MMSE scores of the *f* group were higher than those of the *r* group in the old-old group (≥80 years old) (e). The scent scores of the *f* groups were higher than those of the *r* groups in the young-old (≤69 years old) and old-old groups (f). The hair Mn and V contents were not markedly different between the *f* and *r* groups (g and h). For the frequency of farm work, the MMSE scores of the *f* group were lower than those of the *r* group in the old-old group (i). The scent scores of the *f* group were lower than those of the *r* group in the mid-old group (j). The Mn and Al contents of the *f* group were higher than those of the *r* group (k and l). The plot and bar show the mean value and standard error, respectively. △ and f: frequently at present, □ and *r*: rarely at present. *: $p < 0.05$. **: $p < 0.01$, ns: not significant

8-OHdG/c than those who continued frequent consumption. Furthermore, the K residents who answered that they frequently ate fresh vegetables at present showed higher scores of MMSE and scent identification card test than rare consumers. Fresh vegetables exert various antioxidant effects on cells through vitamins, carotenoids, phytochemicals, phenolics, flavonoids and minerals as well as other essential components for metabolism (23). The K residents with a decreased frequency of fresh vegetable consumption or who rarely ate fresh vegetables at present may have low protection against oxidative stress, and this may be associated with lower scores on cognitive function tests.

The K residents in the 1960s were mainly engaged in farming, fishing, paddy agriculture and forestry. The number of residents in the K area engaged in agricultural and fishing work has decreased to one fifth of that in the 1960s, which was comparable to the demographic data of Kushimoto-cho (24, 25). The products of agricultural pesticides have been found to be dose-dependently associated with ALS in men (26). The decrease in the frequency of agricultural work and an eventual decrease in the usage of agricultural pesticides in the K area may have played a role in the decrease in the ALS incidence in this area.
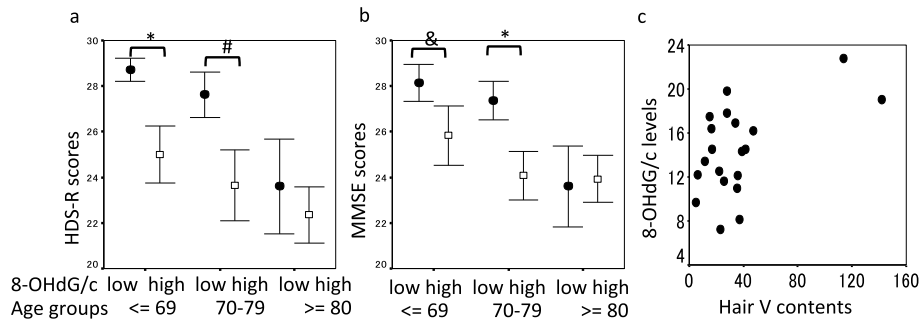
K residents who rarely did farm work both in the 1960s

Figure 6. **The relationships between the cognitive function test scores and oxidative stress among K residents. The HDS-R scores of the low 8-OHdG/c group (<11.8 ng/mg) were higher than those of the high 8-OHdG/c group (≥11.8 ng/mg) in the young-old group (≤69 years old) and in the mid-old group (70-79 years old) (a). The MMSE scores of the low 8-OHdG/c group were higher than those of the high 8-OHdG/c group in the mid-old and young-old group (b). The levels of 8-OHdG/c (ng/mg of creatinine) were positively correlated with the hair V contents (ng/mg wet weight) (Pearson's r=0.56, p<0.05) (c). *: p<0.05, #: p=0.08, &: p=0.1. The plot and bar show the mean value and standard error, respectively. ●: low 8-OHdG/c group, □: high 8-OHdG/c group**

and at present tended to have higher MMSE scores and the scent identification card test scores and had lower contents of Al and Mn in their scalp hair than those who continued to frequently do such work. The K residents who frequently ate Japanese pickles also had higher contents of Mn and V in their scalp hair than those who rarely ate them. Mn, Al and V are widely distributed in the environment, and the levels of Mn and Al in the soil of the K area have been reported to be high (3, 9). Although the mechanisms are not clear, long-term exposure to the soil during farm work and the frequent consumption of Japanese pickles may have been associated with an accumulation of these transition metals in the scalp hair of the K residents. Transition metals exert potent toxic effects on neurons (27, 28) and are associated with a decline in the cognitive ability as determined by the MMSE (29). Given these findings, we speculate that an accumulation of Mn, V and Al may have played a role in the increasing oxidative stress on neurons leading to the deterioration of age-associated cognitive function in vulnerable people of the K area.

In the Hohara area, another high-incidence area of ALS/PDC, Iwami et al. studied the metal contents in the environment between 1989 and 1991 and found that the Mg contents in the drinking water were low and the Mn and Al contents in the food were high via the 24-h total food duplicate method (30). Kuzuhara reported that the epidemiological and clinical features of ALS/PDC in the Hohara area have recently changed, and some unknown environmental factors are speculated to modulate the disease process, some of which may be genetic (7). In this study, we found that the lifestyle of the K residents was recently changed, and we suggest that the lifestyle changes may be associated with the decrease in the oxidative stress and the high scores in the cognitive function test among K residents. The relationships between the lifestyle changes and the epidemiological changes of ALS in the K area should be further investigated

in the future.

The limitations of this study are that the questionnaire used was based on recall of present and past events, and that the sample size was small. The relationships between lifestyle/environment and the risk of ALS need to be examined long-term among both the residents and patients with ALS in the high-incidence areas. In the present study, however, our findings suggest that the recent lifestyle changes in the K area may have helped reduce the oxidative stress on neurons, suggesting that environmental/lifestyle changes may be useful for identifying new therapeutic strategies for not only K-ALS but also sporadic and familial ALS.

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