Dietary Patterns and Risk of Amyotrophic Lateral Sclerosis: A Case-Control Study in Japan

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Abstract: *Objective*: Nearly all epidemiologic studies examining the association between the risk of Amyotrophic lateral sclerosis (ALS) and diet have focused on single foods and specific nutrients. We conducted a population-based case-control study in Japan to examine the relationship between dietary patterns and the risk of ALS.

Methods: The study was comprised of 156 ALS patients and 393 gender- and age- matched controls randomly selected from the general population. At the time of recruitment, dietary intake during the preceding three years before the onset for cases and the interview for controls was assessed using a validated, self-administered food frequency questionnaire. Dietary patterns were extracted by factor analysis.

Results: Three dietary patterns were identified: 'Vegetable-protein based', 'Western' and Traditional-diet' patterns. After adjustment for potential confounding factors, the vegetable-protein based pattern, characterized by a high intake of vegetables, seaweed, pulses, mushrooms, fruits and fish and animal and vegetable protein, was significantly associated with a reduced the risk of ALS (lowest versus highest tertile, adjusted OR 0.35, 95% CI 0.22-0.67). No associations with ALS were detected for the other two dietary patterns.

Conclusion: In this case-control study in Japan, a dietary pattern consisting of high intakes of vegetables, fruits and animal and vegetable protein may be associated with a decreased risk of ALS.

Keywords: Amyotrophic lateral sclerosis, Dietary pattern, Principal component analysis, Nutrition, Case-control study.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease of unclear etiology involving spinal cord motor neurons, leading to atrophy of skeletal muscles, paralysis and rapidly- progressive death. Most the patients are sporadic cases (SALS), while 5-10% of the patients have a family history of ALS (familial ALS or FALS) [1]. Dietary habits and sedentary lifestyles were also rapidly westernized over the past several decades. As for the dietary intake, we have already reported that food rich in antioxidants such as fruit and vegetables [2] and higher intake of fat [3] contribute to protecting against the development of ALS.

Since people do not eat food in isolation, and since food contains many nutrients that work synergically or antagonistically the single eat a complex combination of various dietary antioxidants that may be interactive or synergic, it is difficult to examine their effects separately [4]. In addition, "single nutrient" approach may be also inadequate for considering the combined effect of dietary antioxidants on the risk of ALS should be evaluated. According to Farchi *et al*. [5], analysis based on a large number of nutrients or food items may produce statistically significant associations simply by chance.

Recently, dietary pattern analysis identified by principal components analysis has emerged as an alternative approach because the focus is on foods and nutrients that are consumed in combinations, not alone [4]. Dietary patterns have the two advantages of reflecting the complexity of the dietary habits and overall food intake [6] and to enhance the promotion of healthy food habits [7].

Therefore, we conducted a population-based case-control study to explore the relationship between dietary patterns and the risk of ALS using a relatively large number of patients in Japan.

METHODS

This study method was described previously in detail [2,3].

Briefly, the author has conducted a case-control study of ALS and lifestyle factors. Case subjects were all definite or probable ALS [8] patients aged from 18 to 81 years who had been diagnosed from January 1 2000 to December 31 2004 in the medical centers located in the Tokai area. All cases of progressive bulbar palsy (PBP) were included in this study, but familial progressive muscular atrophy was excluded. There was no evidence of coexisting Parkinson

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disease and related disorders including multisystem atrophy. The present study was approved by the institutional review board of Aichi Prefectural College of Nursing & Health.

The author set up community controls consisting of subjects, matching to each patient for age (±2 years), gender and residence selected by a proportional simple random sampling with stratification by sex and age groups, using the basic register of residents.

A structured self-administered questionnaire specifically designed for this case-control study was distributed and collected by mail in both patients and controls. We asked patients to recall their lifestyle within the 3 years before the onset of ALS, and controls within the 3 years before the survey.

The author asked patients to recall their lifestyle during the three years before the onset of ALS, and community controls the same before their interview. When patients were unable to provide any information on their lifestyle and exposures because of their seriously impaired conditions or early death, proxies (mainly spouses) were interviewed. Standardized in-person interviews were conducted for patients and for their individually matched control. Only when this was not possible was a proxy interview performed. To minimize information bias, when a case's proxy was interviewed, the control's proxy was also interviewed even if the control was competent to be interviewed.

Approval by Aichi Prefectural College of Nursing and Health is in the Study Population and the Data Collection. All participants provided informed consent to a verbal explanation of the study protocol including next of kin for case subjects who were severely ill, unconscious, or dead as well as proxy respondents for control subjects.

A self-administered food frequency questionnaire (FFQ) that included 97 commonly eaten food and beverage items was administered to all participants. Subjects were asked intake frequencies of foods and nutrients with five response options as follows: "never/seldom," "less than once a week," "1-2 times per week," "3-4 times per week," and "almost everyday," and the frequency was dichotomized as "frequent" ("3-4 times per week"/ "almost everyday") vs. "less frequent" ("never/seldom"/ "less than once a week"/ "1-2 times per week.").

Covariates such as demographic characteristic (age at diagnosis, gender) and risk factors were collected with the use of a structured questionnaire specifically designed for this case-control study. Subjects were defined as a physically active if they are active in leisure time for ≥30 minutes at least 3 times per week. Type A behavior pattern was measured by ten-item scale designed for Japanese persons by Maeda [9]. Subjects who scored between 0 and 16 were considered as non-type A, while those who scored 17 or greater were considered as type A. Smoking status was ascertained in relation to number of cigarettes smoked per day during the 5 years before the survey (onset of ALS/interview), and subjects were categorized into current smokers (smoking at least 1 cigarette per day), ex-smokers (not smoking for at least 1 year before the survey), and never smokers, and was classified into current smokers and nonsmokers (including ex-smokers and never smokers). Body mass index was calculated by dividing self-reported body weight (kg) by the square of self-reported height (m).

To identify dietary patterns, the author used principal component analysis, a type of factor analysis in which input variables are aggregated into factors (distinct patterns) by a degree to which they are correlated with one another. We focused on factors whose eigenvalues were greater than 1.30. Food items with a loading of 0.30 or greater were considered to contribute to the factor. Factor scores were categorized into tertiles based on the distribution of the control population.

The odds ratio (OR) and its 95 % confidence interval (C.I.) were estimated by using multiple conditional logistic regression models in which matched variables (gender and age at diagnosis) and potential confounders (type A behavior, body mass index, smoking, drinking) were controlled. [8]

Tests for trend in logistic regression analysis was performed by the exposure variable and treating the scored variables as a continuous one. Statistical analyses were conducted by use of SPSS ver 19.0 (SPSS Japan Inc.).

RESULTS

Among a total of 183 patients with ALS and 407 controls, 156 (85.2 percent) of cases and 393 (96.6 percent) of controls were completed food frequency questionnaire. As shown in Table **1**, no significant difference in mean age and proportion of men was found between cases and controls.

Three dietary patterns that accounted for 42% of the Principal components factor analysis were identified (Table **2**). Principal component 1, which had high

Table 1:   Selected Background Characteristics of Study Subjects

	% or Mean (SD)		p-value
	Cases (n=156)	**Control (n=393)**	
Men (%)	60.3	59.3	p=0.84
Age (years)	63.5(9.3)	63.5(10.6)	p=0.93
Use of proxy respondents (%)	67.5	67.5	p=0.98
Body Mass index (kg/m^2)	22.1(3.2)	23.0(6.0)	p=0.09
Type A behavior pattern (%)	41.6	25.3	p=0.001
Smoking habit (%)	53.9	51.5	p=0.48
Drinking habit (%)	31.8	30.1	p=0.81

Table 2:   Assessment for the Factor-Loading Matrix for Dietary Pattern Identified by Factor Analysis among Controls

	Dietary patterns		
	Vegetable-protein based	**Western**	**Traditional diet**
Rice			0.68
Bread		0.49	
Potatoes		0.32	
Beans	0.39		
Soy products	0.48		
Fish	0.57		0.33
Beef	0.42	0.37	0.34
Pork	0.41		
Chicken	0.45		
Egg	0.46	0.30	
Cheese	0.35	0.33	
Yogurt	0.36		
Batter		0.31	
Mayonnaise	0.31		0.36
Ham/sausage/bacon	0.41	0.38	
Carrot	0.63		
Chinese cabbage	0.69		
Cucumber	0.63		
Tomato	0.52		
Fruit	0.53		
Seaweed	0.57		
Miso soup			0.51
Tsukemono	0.38		0.30
Coffee		0.34	
Proportion of variability (%)	26.0	15.2	5.9

Absolute values <0.30 were excluded from the table for simplicity.

loadings for green and yellow vegetables, seaweed, pulses, other vegetables, mushrooms, dairy products, fruits, fish, soy products, Japanese and Chinese tea, was labeled the Vegetable-protein based pattern. Principal component which had high loadings for bread, meat, ham and sausage, butter and coffee, was

Table 3: **Odds Ratio for Amyotrophic Lateral Sclerosis by Tertiles Based on Scores for Dietary Patterns**

	T1(lowest)	T2	T3 (highest)	P for trend
Factor 1: Vegetable-protein based				
Crude (95%CI)	1	0.48 (0.30-0.76)	0.35 (0.22-0.57)	P=0.001
Multivariate (95%CI) [a]	1	0.47 (0.29-0.77)	0.36 (0.22-0.61)	P=0.001
Factor 2: Western				
Crude (95%CI)	1	0.97 (0.61-1.53)	0.59 (0.37-0.97)	P=0.04
Multivariate (95%CI) [a]	1	1.02 (0.62-1.65)	0.69 (0.42-1.15)	P=0.16
Factor 3: Traditional-diet				
Crude (95%CI)	1	0.71 (0.43-1.18)	1.53 (0.97-2.43)	P=0.06
Multivariate (95%CI) [a]	1	0.71 (0.42-1.21)	1.48 (0.91-2.40)	P=0.10

[a]Odds ratio were separately calculated for each dietary variables adjusted for age, sex, body mass index, smoking habit, drinking habit, and behavior pattern.

labeled the Western pattern. Principal component 3, which had high loadings for rice, miso soup and pickles was labeled the Traditional-diet pattern. The percentages of variation explained were 20.1%, 7.0% and 5.9% for the Vegetable-protein based, Traditional-diet, Western patterns respectively.

As shown in Table **3**, the multivariate-adjusted OR for the highest tertile of Vegetable-protein based pattern was significantly associated with a decreased risk of ALS (lowest versus highest tertile, adjusted OR 0.35, 95% CI 0.22-0.67), implying that those with the highest tertile of vegetable-protein based pattern showed a 65% reduction in risk of ALS. No significant relationship between Traditional-diet and Western pattern and the risk of ALS was observed.

DISCUSSION

The author found that the vegetable-protein based dietary pattern only was significantly associated with a decreased risk of ALS, but Traditional diet and Western pattern had no significant relationship after adjustment for covariate factors. This is the first epidemiological finding that a plant -based dietary pattern including fish and poultry may have a protective role in the onset of ALS.

A methodological issue is that FFQ was used to obtain information on dietary habit. The reproducibility of food frequency questionnaire that we used in this study showed moderate, with a kappa-statistic ranged from 0.45 to 0.63 over a 1-month interval among 50 community controls subjects. Moreover, Drewnowski *et al*. reported mean frequencies of food consumption were a significant predictor of dietary outcomes [10]. In addition, we used a self-administered questionnaire to

obtain retrospective information on dietary habits. Therefore, the reliability of the self-administered questionnaire was assessed using the test-retest over two week periods for twelve persons randomly selected among community controls. Kappa values ranged from a low of 0.52 for vegetables to a high of 0.86 for rice, suggesting that the measures in this study might be considered adequately reliable.

In this study, the author found that the vegetable-protein based dietary pattern including various foods such as meat, fish, egg, soy products, green and yellow vegetables and fruits was significantly decreased with the ALS risk. This finding was consistent with the author's results that dietary intake of fruits and vegetables [2] and much intake of fat and protein [3] were a significant association with a decreased risk of ALS. Niebroj-Dobosz *et al.* [11] experimentally examined spinal cords of 8 sporadic ALS cases and 5 control proposed the necessity of an anti-oxidative neuroprotective strategy in ALS because ALS results from oxidative damage to spinal cord proteins. Fruits and vegetables are rich sources of antioxidant vitamins and minerals such as vitamin C and E, beta-carotene, potassium, magnesium, and calcium [12]. It is known that the increase of intake of fruit and vegetables may protect against oxidative stress as a free radical scavenger system [11,13]. These findings could be a supportive explanation as to the underlying mechanism for observed the relationship between fruits and vegetable intake and the risk of ALS. Moreover, vegetable-protein based dietary pattern consisted of animal and vegetable protein such as fish, soybeans respectively. These finding agreed with the author's previous result that the percentage of total energy to protein had significantly associated with a decreased risk of ALS [3]. The inverse association

detected between the vegetable-protein based dietary pattern and risk of ALS can be explained by the cumulative effects of individual nutrients, implying that well-balanced meal due to a combination of food high in antioxidants vitamin, and animal and vegetable protein has protective against the development of ALS. Moreover, the author has previously reported that much intake of carbohydrate and less intake of fat and protein were significantly associated with an increased risk of ALS [3]. In this study, Traditional-diet pattern was not significantly associated with an increased risk of ALS, although the loading of rice was the highest among food items consisting of the dietary pattern. These findings indicate that dietary foods and nutrients are not eaten in isolation, and those may have interactive or synergistic effects of a combination of health rather than any one food.

There are several limitations to this study. First, the author used prevalent patients whose diagnosis was made within 1-4 years before the present study, which would result in some difficulty in recalling their conditions before the onset of ALS. It is quite likely that prevalent cases often take the influence of the recall bias in comparison with incident cases. According to Swan *et al*. [14], it is difficult to assess reporting bias because of the absence of a reference criterion against which to compare self-reported, retrospectively collected information in case-control studies. In this study, the association of the studied variables and the risk of ALS remained even after we reanalyzed for the subjects within one year before the onset of ALS, although the number of cases were small (n=64).

Second, our questionnaire asked several information 3 years before recruitment into study. This was because the author examine the causality between lifestyle factors before the onset of ALS and the risk of one. Accordingly, the possibility could arise that lifestyle exposure might have occurred after initiation of ALS development. Therefore, the author have confirmed that all of cases and controls had no significant change in their lifestyle between 3 years at interview and 10 years prior to disease onset. Reliability and validity on information collected among latent interval for symptom to appear have been pointed out as a methodological issue in research that involves interviews or questionnaires as case-control studies.

To avoid these problems, therefore, we are now planning relatively large population- based prospective study. Third, the author used the self-administered questionnaire to collect information both cases and controls. The authors have demonstrated no significant difference in the responses to questions related to lifestyle factors such as physical activity, general life stress and dietary habit between self- and interviewer-administered questionnaires [15]. Marshall *et al*. reported that 90% of the estimates by spouses and by respondents to food-frequency questionnaires are within one frequency category of each other [16]. In our study, associations between macronutrients and ALS occurrence remained after excluding the data obtained from proxy respondents (data not shown). These findings suggest that the effect of our collection method on subjects' responses would be minimal. Fourth, in this study, the author examined based on information of food frequency within the 3 years before the onset of ALS, although it is highly likely that kind or degree of severity of chief complainants such as muscle weakness and difficulty swallowing at onset affect dietary intake. Therefore, the author need to collect that information to provide more detail information on the manner of food intake against the development of ALS.

Our current investigation had methodological strengths that were identified according to the most recent diagnostic criteria, and adjustment was made for extensive potential confounders.

In summary, the vegetable-protein based pattern was associated with a reduced risk of ALS. This pattern can be considered as a well-balanced dietary pattern for Japanese. Further interventional or longitudinal studies are necessary to confirm our finding.

ACKNOWLEDGEMENTS

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