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Abstract

Recent progress in ALS research: ALS and TDP-43

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Selective involvements of upper and lower motor neurons have been regarded as one of the most characteristic features of amyotrophic lateral sclerosis (ALS). However, evidences of more extensive involvements affecting the systems other than the pure motor systems have been accumulated since the discovery of ubiquitin-positive inclusions (UbIs) in ALS, ALS-dementia (ALS-D), and frontotemporal lobar degeneration (FTLD) with UbIs (FTLD-U). A breakthrough occurred in ALS research in October 2006, when TAR DNA-binding protein-43 (TDP-43) was identified as the core protein that is ubiquitinated in the cytoplasm, neurites and nucleus as UbIs. Antibody to phosphorylated TDP-43 selectively reacts to the inclusions and Western blotting demonstrates abnormal bands of phosphorylated TDP-43 in the brains of patients with ALS/FTLD-U. Similar findings were observed in ALS/parkinsonism-dementia complex (PDC) of Guam and Kii peninsula. These diseases are lumped in the "TDP-43 proteinopathy". In early 2008, several mutations of the *TDP-43 gene* were identified as the causative gene of autosomal-dominant familial ALS without *SOD1 gene* mutations. These findings suggest that abnormalities of TDP-43 directly or indirectly produce severe motor neuron degeneration. TDP-43 is thus one of the key proteins causing TDP-43 proteinopathies such as ALS, ALS-D, FTLD-U, and ALS/PDC of Guam and Kii. New revolutionary developments on ALS research for molecular mechanism and therapy are expected.

(Clin Neurol, 48: 625—633, 2008)

Key words: ALS, TDP-43, frontotemporal lobar degeneration, ubiquitin, familial ALS

神経内科，68(6)：565-570，2008

特集 TDP-43と神経変性疾患

紀伊半島ALS/PDCとTDP-43*

葛原茂樹**

Key Words：TDP-43, ALS, parkinsonism-dementia complex, Kii peninsula, immunohistochemistry

紀伊半島の熊野灘に沿った山岳地帯は牟婁(むろ)地方と呼ばれ，グアム島と並んで筋萎縮性側索硬化症(ALS)の高集積地が点在する．グアムと紀伊のALS集積地には，Parkinson症状と認知症の組み合わせを主徴とする特異な疾患であるParkinson認知症複合(parkinsonism-dementia complex：PDC)も多発する．ALSとPDCは同じ集落内に高集積しているのみでなく，しばしば同一家系内あるいは同一患者にも両疾患が出現するので，一つのスペクトル上にある疾患という概念から，西太平洋地域ALS/PDCと呼ばれる[1]．

ALS/PDCのもっとも特異な神経病理学的特徴は，老人斑を伴うことなく大脳皮質(とくに辺縁系と側頭葉，前頭葉)，視床下部，脳幹，脊髄の中枢神経系にAlzheimer神経原線維変化(NFT)が広汎かつ大量に出現することであり，一次性タウタンパク異常症(tauopathy)と考えられてきた[2]．

一方，孤発性ALS[3]とPick球が認められない(非タウ性の)前頭側頭葉変性症(frontotemporal lobar degeneration：FTLD)[4]には海馬歯状回や被殻の神経細胞質内あるいは核内に，ユビキチン陽性の封入体が出現し，motor neuron disease-inclusionと呼ばれてきた．2006年にNeumannら[5]，Araiら[6]は，ALSとFTLDの中枢神経に出現するユビキチン陽性封入体の主要構成要素がTAR DNA-binding protein of 43 kDa(TDP-43タンパク)であることを初めて報告した．さらにHasegawaら[7]は，グアムのPDCにもTDP-43陽性封入体が出現することを報告した．著者らは，紀伊半島のALS/PDCの脳と脊髄においてTDP-43免疫組織化学を実施し陽性封入体を認めた[8]ので，この所見を中心に紹介する．

西太平洋地域ALS/PDCの神経病理学的所見

既に報告した通り[9][10]，紀伊ALSでは，典型的なALS病変以外に，NFTがさまざまな程度にみられることが特徴である．大脳の肉眼的萎縮は認められないことが多いが，NFTは後述のPDCと同じように広汎に出現する．しかし，数は非常に多いものから比較的少数にとどまるものまで症例によってバラツキがある．紀伊PDCの病理所見の特徴は，グアム例と紀伊半島例で相違はなく，前頭葉と側頭葉に先端部優位の肉眼的萎縮があり，組織学的にNFTが多数かつ広範に出現するが，対照的にアミロイド老人斑がほとんどみられない[9][10]．NFTの分布は，皮質表層のII～III層に顕著であるのが特徴で，深層の方に顕著なAlzheimer病(AD)における分布とは異なる[11]．PDC症例では，臨床的な運動ニューロン症状の有無に関係なく，ALS病変と同質の上位と下位の

* TDP-43 in ALS/parkinsonism-dementia complex of the Kii peninsula.

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表1　6症例のまとめ

家系	臨床診断	脳重（g）	ALS病変 上位／下位	NFT 海馬／脊髄	TDP-43 海馬／脊髄
H	ALS	1,190	3＋／3＋	1＋／±	2＋／2＋
F	ALS	1,275	1＋／1＋	3＋／±	2＋／2＋
A	D＋ALS	1,300	－／2＋	3＋／1＋	3＋／1＋
A	PDC	935	1＋／2＋	3＋／1＋	3＋／2＋
D	PDC	1,085	1＋／2＋	3＋／2＋	1＋／1＋
A	PDC＋ALS	960	2＋／3＋	3＋／1＋	3＋／1＋

＊家系の同一アルファベットは同一家系の出身を示す．
＊臨床診断．ALS：ALS症状のみが認められたもの．D＋ALS：認知症が先行したALS．PDC：パーキンソニズムと認知症のみが認められたもの．PDC＋ALS：Parkinson認知症複合の症状の経過中にALS症状が加わったもの．3＋：顕著，2＋：中等度，1＋：軽度，±：痕跡，－：なし．

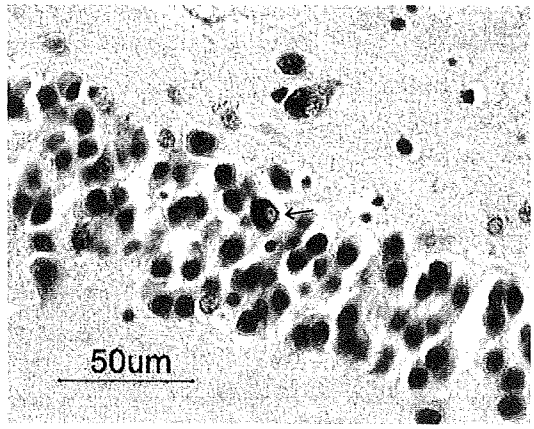

図1　紀伊ALS症例海馬歯状回のTDP-43免疫組織化学染色
TDP-43陽性封入体のある細胞（矢印）では，核が染色されない．正常細胞の核は茶色に免疫染色されている．

運動ニューロン変性が高頻度に認められる[9]．ALS，PDCともに，一部の症例ではHE染色でも明瞭に判別できるLewy小体が脳幹とMeynert基底核，扁桃体に出現する．

TDP-43

TDP-43は核蛋白の一種で普遍的にみられ，転写抑制因子およびエクソンスキッピング活性化因子として働いている．2006年にNeumannら[5]，Araiら[6]によって，このタンパクがFTLDとALSの海馬歯状回に出現するユビキチン陽性封入体の主要な構成要素であることが明らかにされ，大きな注目を浴びるところとなった．海馬歯状回の正常神経細胞の核は免疫組織学的にTDP-43陽性であるが，細胞質内あるいは核内にTDP-43陽性封入体を認める神経細胞の核は，抗TDP-43抗体で染色性を喪失していることも示された．抗TDP-43抗体は抗ユビキチン抗体よりも鋭敏でより多くの封入体を標識し，歯状回，前頭葉や側頭葉の大脳皮質の神経細胞，変性したneuriteのみでなく，白質のグリア細胞内（主として乏突起細胞）にも陽性封入体が出現する[12]．また，孤発性ALSの脊髄前角細胞内のskein-like inclusionやround inclusionもTDP-43陽性である．新鮮脳のウェスターンブロットでは，FTLDとALSでは43kDに共通の帯が認められ，その他に45kDの小帯とスメア様の淡い陽性所見が認められている．

抗TDP-43抗体が認識する封入体の微細構造も，金粒子を結合させた抗体を用いた免疫電顕によって示されている．Amador-Ortizら[12]は，金粒子を結合させた抗TDP-43抗体によってAD症例の核内および細胞質内のTDP-43陽性封入体を免疫電顕的に標識し，金粒子は顆粒で覆われた線維構造に結合していることを確認した．

このように，孤発性ALSと，従来はPick球を欠くFTD，非タウFTLD，ユビキチン陽性封入体FTDなどの名称で呼ばれていた運動ニューロン変性を伴うFTLDが，TDP-43 proteinopathyという一つの疾患概念にまとめられた[14]．今や，TDP-43はアミロイド，タウ，α-シヌクレイン，プリオンに次ぐ第4番目の中枢神経蓄積異常蛋白の座を占めることになった．

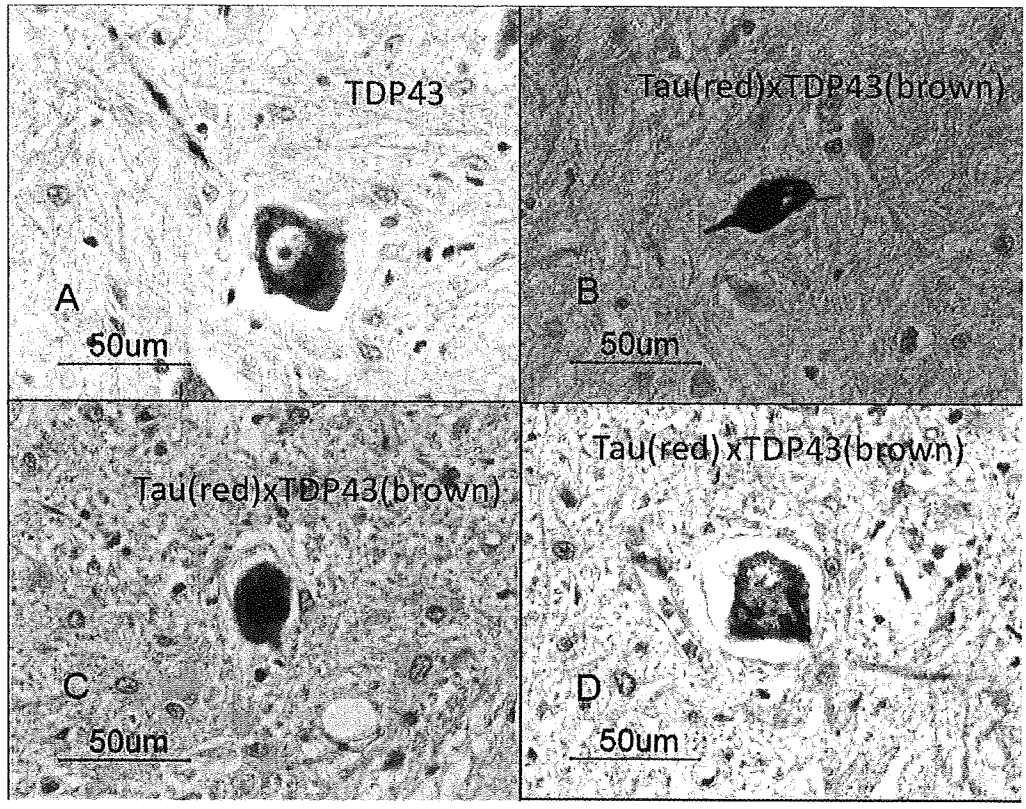

図2　紀伊ALS例の脊髄タウ蛋白(赤)とTDP-43(褐色)の二重免疫染色
TDP-43陽性の細胞質内と突起内の小顆粒状(A)，大顆粒状(C)，skein-like inclusion(D)とタウ蛋白陽性の細胞質内神経原線維変化(B)．二重免疫組織化学では，これらの細胞で，見る限りにおいてTDP-43とタウタンパクは共存していない．

紀伊半島と西太平洋地域のALS/PDCにおけるTDP-43

著者ら[15]は，紀伊ALS/PDCの6例の側頭葉と脊髄のホルマリン固定・パラフィン包埋切片において，抗TDP-43抗体(モノクローナル抗体：TRDBP monoclonal antibody(MO1)，Abnova Co.（Taiwan)，ポリクローナル抗体：Antibody to TARDBP(BC001487)，Protein Tech Group, Inc.，Chicago, IL, 1,000倍希釈)を用いて免疫組織学的に検討した．併せて，抗リン酸化タウ抗体(AT8)，抗アミロイドβ抗体：Aβ11-28(12B2)，抗ユビキチン抗体，抗リン酸化α-シヌクレイン抗体(東京大学・岩坪　威博士より供与)も実施した．

得られた6症例の免疫組織化学的所見を，患者の臨床的病理学的背景とともに表1に示す．TDP-43陽性封入体は，臨床病系がALSであったかPDCであったかを問わず，海馬歯状回と脊髄前角細胞に認められた．

図1，2に示す代表的症例は，臨床表現型は上肢遠位部の筋萎縮から始まった古典型ALSで，パーキンソニズムや認知症を出現することなく，全経過5年で死亡した63歳女性例の脳と脊髄の所見である．脳重1,140gで，上位と下位の運動ニューロンの変性に加えて，脳全体に多数のNFTが認められた．タウ免疫組織化学では，脳全体に多数のNFTとタウ陽性神経突起を認め，脊髄前角細胞内にもNFTが認められた．

側頭葉のTDP-43免疫組織化学(図1)では，海馬歯状回神経細胞内封入体を認め，このような細胞では核の染色性は失われていた．核内封入体は見出せなかった．脊髄(図2)では，前角細胞のskein-like inclusionが陽性であり，その他に細胞質内に顆粒状，小塊状，あるいは円形の陽性封入体を認めた．

[新鮮凍結脳材料のウェスタンブロット]

既にFTDやALSで報告されているものと同じく，正常例で認められる43kDaのバンドのほかに，紀

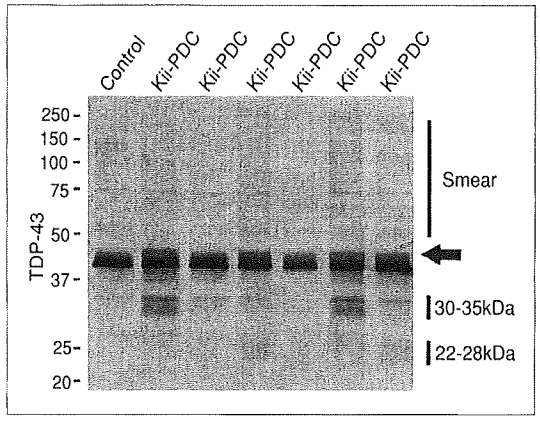

図3　新鮮凍結脳のウェスタンブロット
抗TDP-43抗体では，コントロールを含めて43kDaに濃いバンドが認められる．さらに紀伊ALS/PDC例では，さらに45kDaに別のバンド(←)が認められ，そこから淡いスメア状の反応が認められる．

伊ALS/PDC症例では45kDa の部分に異常なバンドが出現し，さらにそこからスメア状に淡い反応が認められた(図3)．

グアムのPDC例について，Hasegawaら[7]もまったく同じ知見を報告している．したがって，紀伊とグアムのALS/PDCはtauopathyであると同時にTDP-43 proteinopathyでもある．

この知見が加わったことによって，NFT多発やtauopathyのみでは説明困難であった運動ニューロン病としての生化学的側面を，合理的に説明できるようになった．つまり，紀伊とグアムのALS/PDCは，生化学的にはタウ蛋白とTDP-43が蓄積し，しかもβアミロイド沈着を伴わない独特の神経変性疾患とみなすことができる(図4)．原因については，紀伊ALS/PDC症例にはタウ蛋白，progranulin, TDP-43の遺伝子には変異は認められなかった(Tomiyama H, et al, in submission)．局所的に高集積している根本原因が遺伝子異常か環境因か，それとも両者の相互作用によるものかは今後の検討課題である．

その他の疾患とTDP-43

研究が進むにつれて，FTLD, 孤発性ALS, 紀伊やグアムのALS/PDC以外の疾患においてもTDP-43蓄積が報告されるようになった．まず，FTLD-MND-ALSの範疇に属す疾患として，*proguranulin*遺伝子変異によるFTLD[5,16]と，*valosin-containing protein*(*VCP*)遺伝子変異によるFTD(封入体筋障害と骨のPaget病を伴う)[17]において，TDP-43タンパク異常が確認されている．家族性ALSの中で，*SOD1*遺伝子変異を伴わない優性遺伝ALSではTDP-43タンパク異常が認められたが，もっとも頻度の高い*SOD1*遺伝子変異によるALSでは認められなかった[18]．2008年2月にSreedharanら[19]は，男性から男性へと優性遺伝する英国系白人家系のALSにおいて，TDP-43をコードする遺伝子である*TARDBP*のexon 6にM337Vの変異を報告し，TDP-43異常によって直接に運動ニューロンが障害されることが明らかになった．彼らはさらに，孤発性ALS例における*TARDBP*変異を英国人200例とオーストラリア白人172例について調べ，Q331KとG294Aの変異

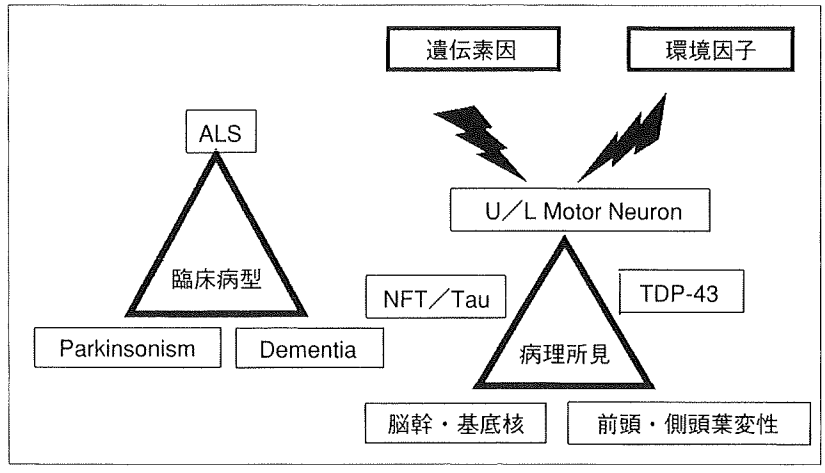

図4　紀伊ALS/PDCの概念，臨床病型，病理所見と病的生化学，病因のイメージ

をそれぞれに1例ずつに見出した．2008年4月にはGitchoら[20]がexon 6にA315Tの変異家系を，Yokosekiら[21]は剖検でBunina小体とTDP-43陽性封入体が確認されている家系において，exon 6のA1028G(Q343R)の変異を報告した．TDP-43をコードする遺伝子変異によるALS発症が示されたことにより，今後はこの遺伝子変異が選択的運動ニューロン障害をひき起こすメカニズムの研究が進むことが期待される．

この他にも，Amador-Ortizら[12]は，海馬歯状回と海馬傍回におけるTDP-43陽性封入体の出現を，ADの20%，海馬硬化症の70%に，Nakashima-Yasudaら[22]はAD病変を伴うLewy小体型認知症(DLB)の31%，PDの7%，PD認知症(PDD)の19%に認めたことを報告した．この所見の意味，すなわちTDP-43封入体の出現が，疾患独自の変化か，二次的変化か，それともFTLDの合併であるのかについては，今後の研究成果に俟ちたい．

おわりに

筋萎縮性側索硬化症(ALS)/Parkinson認知症複合(PDC)はグアムと紀伊半島の限局した地域のみに多発し，ALS,パーキンソニズム，認知症という三つの臨床表現型をもち，それらの組み合わせの症状が出現する．神経病理学的・神経化学的に特異な点は，アミロイド蓄積を伴うことなく神経細胞内にリン酸化タウ蛋白が異常蓄積することで典型的tauopathyと考えられていた．しかし，少数例の見当ではあるが全例の海馬にTDP-43陽性封入体が出現しており，脊髄ではALSに特異的なskein-like inclusionやround inclusionもTDP-43陽性であったので，現時点では"combined tauopathy and TDP-43 proteinopathy"と見なすべきであり，その方が臨床像と病理象を合理的に説明できる．このような異常タンパクが蓄積する仕組みと原因は，今後の研究課題である．

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Fruit and Vegetable Intake and Risk of Amyotrophic Lateral Sclerosis in Japan

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Key Words
Amyotrophic lateral sclerosis · Antioxidants · Fruit · Vegetables

Abstract
Background: There has been little interest in the role of nutrition in the prevention of amyotrophic lateral sclerosis (ALS). We investigated the relationship between dietary intake of vegetables, fruit, and antioxidants and the risk of ALS in Japan. ***Methods:*** Between 2000 and 2004, we recruited 153 ALS patients aged 18–81 years with disease duration of 3 years within the study period in accordance with El Escorial World Federation of Neurology criteria. Three hundred and six gender- and age-matched controls were randomly selected from the general population. Information on dietary factors was collected using a validated self-administered diet history questionnaire. ***Results:*** A higher consumption of all fruits and vegetables and fruit alone in the highest quartiles was associated with a statistically significantly reduced risk of ALS. Although not statistically significant, a beneficial association between intake of all vegetables, green and yellow vegetables and other vegetables and ALS was found. No statistically significant dose-response relationship was observed between intake of β-carotene, vitamin C and vitamin E and the risk of ALS. ***Conclusion:*** Our findings suggest that higher intake of food rich in antioxidants such as fruit and vegetables confer protection against the development of ALS.

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 rapid progression to death. The majority of the patients are sporadic cases, while 5–10% of the patients have a family history of ALS [1]. Dietary habits and sedentary lifestyle were also rapidly westernized over the past several decades. Several epidemiological studies have examined the risk factors of ALS; most have focused on physical activity [2–4], skeletal fracture [5] and heavy metal exposure at work [6–8].

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ALS was previously found to be positively associated with intake of glutamate [9], fat [10], fish [11] and milk [11, 12], and inversely associated with intake of lycopene [13], dietary fiber [9], bread and pasta [14]. Moreover, we have previously demonstrated that a high intake of carbohydrate was positively, and high intakes of total fat, saturated fatty acid, monounsaturated fatty acid and polyunsaturated fatty acid were inversely associated with a decreased risk of ALS, suggesting that nutrients may be factors that could influence these processes and thereby the risk of developing ALS [15].

Free radical accumulation and oxidative stress have been proposed as contributing to the progression of ALS (or motor neuron disease). A few epidemiological studies have examined the relationship between dietary intake of antioxidant vitamins and the risk of ALS; most have focused on intake of vitamin E [16–18]. However, the results have been inconsistent. As far as we know, there is no epidemiological information about the relationship between food rich in antioxidants and the risk of ALS. In this study, we focused on fruits and vegetables, as they are rich sources of antioxidant nutrients. We examined the relationship between dietary intake of fruits and vegetables and the development of ALS using a case-control study in a Japanese population.

Methods

Study Populations

We conducted this study in medical centers in the Tokai area of Japan from 2000 to 2004, using a self-administered questionnaire. Cases were all definite or probable ALS patients, who were under the care of experienced neurologists and met the El Escorial World Federation of Neurology criteria [19], aged 18–81 years with disease duration of 3 years or less within the study period. All cases of progressive bulbar palsy were included in this study, whereas familial progressive muscular atrophy was excluded. There was no evidence of coexisting Parkinson disease or related disorders including multisystem atrophy.

We set up two community controls matched to each patient for age (±2 years), gender and residence based on electoral districts. They were selected by a proportional simple random sampling from among the general population in the same district as our case subjects based on the basic register of residents. A total of 612 eligible controls were contacted and 505 (82.5%) were enrolled in this study; 418 completed the entire questionnaire.

Data Collection

Subjects were asked to report their behavior and characteristics 3 years before the time of the diagnosis, using a mailed self-administered questionnaire. To facilitate the recall of lifestyles, including dietary habits, we inserted into the questionnaire some questions about their familial and social backgrounds at that time.

Institutional ethics committees of the Aichi Prefectural College of Nursing and Health approved the protocol before commencement.

Dietary Information

A self-administered food frequency questionnaire (FFQ) that included 97 commonly eaten food and beverage items was administered to all participants. This FFQ was validated in a healthy Japanese population using a 16-day dietary record as a standard, and was proven to be adequately valid for most of the nutrient and food groups [20, 21]. For each fruit or vegetable, a standard unit or portion size was specified and participants were asked how often, on average, during the previous month they had consumed that amount 3 years before recruitment into study. Seven responses were possible, ranging from 'never' to 'six or more times per day'. The energy-, sex- and age-adjusted test-retest correlation coefficients between the two FFQs administered at a 1-year interval ranged from 0.34 to 0.78 (median, 0.57) for the nutrients under study. The deattenuated, energy-, gender- and age-adjusted correlation coefficients between the second FFQ and the dietary records were larger than 0.40 for most food groups. Nutrient intake from supplements was not considered in the present study. Estimates of nutrient intake were computed by using the Standard Tables of Food Composition in Japan, fifth revised and enlarged edition (Science and Technology Agency, 2000).

Covariates such as demographic characteristics (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 physically active if they were active for ≥30 min for at least 3 times a week in their leisure time. Smoking status was ascertained in relation to the number of cigarettes smoked per day during the 5 years before the survey, 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 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 (cm).

Statistical Analysis

The differences in mean values or frequencies between ALS patients and controls were statistically examined by the unpaired t test, χ^2 test, or Mantel-Extension test. The odds ratio (OR) and its 95% confidence interval (CI) were estimated by using multiple conditional logistic regression models to access the strength of association between ALS and potential risk factors [17]. For each nutrient or micronutrient of interest, the 25th, 50th, and 75th percentiles of the control group distribution were used to define four quartile-based categories of intake. ORs were calculated for each nutrient by using the lowest quartile of intake as the referent group. Tests for trend in logistic regression analysis were performed by the exposure variable and treating the scored variable as a continuous one. All nutrient analyses were adjusted for total amount of food consumption for fruit and vegetables and energy intake using the residual method for β-carotene and vitamins. All logistic regression models used to estimate the effects of nutrient intake included the following covariates: behavior pattern, smoking status, and body mass index.

In the analysis of estimated nutrient intakes, all the nutritional variables were natural logarithmically transformed to improve their normality. Because intake of most nutrients is strongly correlated with total energy intake, the former was adjusted for the latter using the residuals from linear regression models. For this analysis, subjects were divided into three groups according to the quartile of energy-adjusted nutrient intakes among controls.

The latency period for ALS may be longer than a few years. To address the possibility that change in lifestyle due to potential progression of ALS might have affected the results, we asked whether they changed their lifestyle including dietary habits from 3 years before the onset of ALS to the date of the study. We excluded participants with change in lifestyle, extreme daily energy intakes (<800 or >4,000 kcal for men and <500 or >3,500 for women) or incomplete FFQ.

Results

Of the total number of 254 patients within the study period, 194 cases (76.4%) who met the inclusion criteria were included in this study. Of the 194 cases, 31 did not respond, resulting in 163 eligible cases of whom 153 completed the entire questionnaire. Table 1 shows the characteristics of cases and controls. The mean ages were around 63.0 years, accounting for about 60% of the men among ALS patients and community controls. The proportion of proxy interviews was similar between ALS patients and controls.

Table 2 shows the relation between vegetable and fruit consumption and ALS risk. We observed a significantinverse association between higher intake of all fruits and vegetables (p for trend = 0.04) and all fruits (p for trend = 0.02) and ALS risk in the multivariate model. In the multivariate model, an inverse association was observed between intake of all vegetables, green and yellow vegetables and ALS, although this trend was not statistically significant. The inverse relationship between intake of other vegetables and the risk of ALS was significant after controlling for sex and age (p for trend = 0.06), but completely disappeared in the multivariate model.

Table 3 presents adjusted ORs for ALS in relation to β-carotene, and vitamins C and E. A higher intake of vitamins C and E was associated with a reduced risk of ALS, but there was no statistically significant association. There was a significant inverse relationship between β-carotene intake and the risk of ALS by the multivariate model: the ORs for the highest versus lowest quartile were 0.66 (95% CI, 0.35–1.23; p for trend = 0.04).

Table 1. Selected background characteristics of study subjects

	Cases (n = 153)	Control (n = 306)	p value
Sex, %			
Men	60.3	60.3	
Women	39.7	39.7	
Age group, %			
–49	32.6	33.3	
50–59	36.4	34.8	
60–	31.0	31.9	
Age (mean ± SD), years	63.7 ± 9.2	63.4 ± 10.6	NS
Use of proxy respondents, %	67.5	67.5	NS
Body mass index (mean ± SD)	22.2 ± 0.2	23.3 ± 0.3	0.04
Type A behavior pattern, %	44.2	19.6	0.000
Smoking habit, %	58.1	54.1	NS
Drinking habit, %	35.3	31.4	NS
Energy intake, kcal/day			
<1,554	32.7	24.5	
1,554–1,987	20.5	24.6	
1,987–2,418	21.6	24.3	
>2,418	25.1	25.6	0.03

NS = Not significant.

Discussion

In the case-control study of ALS, we found that higher intake of all fruits and vegetables and fruit alone was significantly associated with a reduced risk of ALS. As far as we know, no epidemiological information is available about the relationship between antioxidant-rich foods and the risk of ALS. This is the first epidemiological finding that a high intake of all fruits and vegetables as antioxidant-rich foods may be protective against the onset of ALS. We also observed statistically significant reductions in ALS risk (approximately 50%) in the highest category of intakes of fruits and vegetables compared with the lowest, and the inverse relationship remained in the multivariate model.

A methodological issue may be that we gathered information at a time 3 years prior to the onset of the diagnosis. In case of ALS patients, it is quite likely that patients' recall may have been influenced by their diet after the onset of the disease due to its related symptoms such as difficulty in swallowing and muscular weakness. Accordingly, it is difficult to determine whether they reported the pre-illness dietary intake 3 years before the onset. Therefore, we did our best to accurately assess dietary intake before the onset of the disease using a validated

Table 2. ORs (95% CI) for ALS by quartiles of vegetable and fruit intake

Variables	Quartile of intake				
	1	2	3	4	p for trend
All fruits and vegetables					
Cases/controls	39/76	38/75	40/77	36/78	
Sex and age adjusted	1.00	0.82 (0.50–1.35)	0.66 (0.40–1.10)	0.57 (0.34–0.97)	0.03
Multivariate adjusted[1]	1.00	0.92 (0.55–1.56)	0.77 (0.45–1.32)	0.61 (0.39–1.29)	0.04
All fruits					
Cases/controls	40/78	36/74	37/77	40/77	
Sex and age adjusted	1.00	0.81 (0.49–1.33)	0.73 (0.44–1.22)	0.51 (0.30–0.87)	0.008
Multivariate adjusted	1.00	0.83 (0.50–1.38)	0.79 (0.47–1.34)	0.57 (0.33–1.01)	0.02
All vegetables					
Cases/controls	38/77	37/75	39/78	39/76	
Sex and age adjusted	1.00	0.89 (0.53–1.49)	0.92 (0.55–1.54)	0.77 (0.45–1.31)	0.37
Multivariate adjusted	1.00	0.95 (0.55–1.63)	0.98 (0.57–1.69)	0.80 (0.44–1.44)	0.51
Green and yellow vegetables					
Cases/controls	38/78	39/74	37/77	39/77	
Sex and age adjusted	1.00	0.99 (0.58–1.72)	1.11 (0.65–1.89)	0.80 (0.52–1.57)	0.51
Multivariate adjusted	1.00	0.95 (0.54–1.98)	1.09 (0.62–1.91)	0.83 (0.50–1.61)	0.69
Other vegetables					
Cases/controls	38/77	37/75	39/78	39/76	
Sex and age adjusted	1.00	0.53 (0.32–0.88)	0.89 (0.54–1.45)	0.50 (0.30–0.85)	0.06
Multivariate adjusted	1.00	0.61 (0.35–1.05)	1.12 (0.65–1.93)	0.63 (0.35–1.13)	0.43

[1] ORs were separately calculated for each dietary variables adjusted for age, sex, body mass index, smoking habit, drinking habit and behavior pattern and total energy.

Table 3. ORs (95% CI) for ALS by quartiles of β-carotene and vitamins C and E intake

	Quartile of intake				
	1	2	3	4	p for trend
β-Carotene					
Cases/controls	40/78	36/74	37/77	40/77	
Sex and age adjusted	1.00	1.24 (0.76–2.05)	0.81 (0.47–1.39	0.69 (0.40–1.21)	0.08
Multivariate adjusted[1]	1.00	0.92 (0.63–2.03)	0.78 (0.44–1.38)	0.66 (0.35–1.23)	0.04
Vitamin C					
Cases/controls	39/76	38/75	40/77	36/78	
Sex and age adjusted	1.00	0.71 (0.42–1.20)	0.77 (0.45–1.30)	0.84 (0.50–1.41)	0.57
Multivariate adjusted	1.00	0.73 (0.43–1.25)	0.78 (0.45–1.36)	0.83 (0.46–1.47)	0.59
Vitamin E					
Cases/controls	38/77	37/75	39/78	39/76	
Sex and age adjusted	1.00	0.67 (0.40–1.14)	0.60 (0.35–1.05)	0.89 (0.61–1.63)	0.96
Multivariate adjusted	1.00	0.71 (0.40–1.26)	0.66 (0.34–1..27)	0.99 (0.54–2.42)	0.78

[1] ORs were separately calculated for each dietary variables adjusted for age, sex, body mass index, smoking habit, drinking habit and behavior pattern and total energy.

FFQ and, to facilitate the recall of lifestyles, including dietary habits 1 year previously, some reminder questions were inserted into the questionnaire regarding familial and social backgrounds at the time. Nevertheless, it is difficult to ascertain whether the questionnaire correctly gathered information reflecting a time 3 years prior to the study. Prospective studies are warranted to confirm the associations between diet and ALS risk. However, in a low-incidence area like Japan, such studies are extremely challenging to perform. Hence we have initially conducted a case-control study.

It has been suggested that ALS, a neurodegenerative disorder resulting in motor neuron death, is associated with oxidative damage induced by free radicals. Niebroj-Dobosz et al. [22] proposed the necessity of an antioxidant neuroprotective strategy in ALS because ALS results from oxidative damage to spinal cord proteins. In our study, we observed that fruit and vegetable intakes were significantly associated with a lower risk of ALS. Fruits and vegetables are rich sources of antioxidant vitamins and minerals such as vitamins C and E, β-carotene, potassium, magnesium, and calcium [23]. It is known that the increase in intake of fruits and vegetables may protect against oxidative stress as a free radical scavenger system [24, 25]. These findings could be a supportive explanation for our results that showed a relationship between fruit and vegetable intakes and ALS risk.

However, we observed that dietary intake of green and yellow vegetables, other vegetables, and antioxidant vitamins (β-carotene, vitamins C and E) were associated with a reduced risk of ALS, although not statistically significant, implying that these foods and nutrients are protective against the development of ALS. In general, dietary antioxidants are not eaten in isolation, and those may have interactive or synergistic effects on health when combined. This may be because the mixture of antioxidants contained in fruits and vegetables and antioxidant vitamins has a greater effect than a single food and antioxidant vitamin alone. Accordingly, these findings suggest that the development of ALS may be inhibited by synergic and interactive effects of individual dietary foods and antioxidants.

Consistent with previous studies [26, 27], our results found that a diet rich in fruits and vegetables is associated with a lower proportion of smoking and drinking habit, body mass index, and psychological stress (data not shown). However, adjustment for potential confounders of ALS such as age, sex, body mass index, smoking habit, drinking habit and behavior pattern and total energy did not alter the protective association of fruit and vegetable intakes, suggesting that all fruit and vegetable intakes may have independent effects on ALS prevention.

There are several limitations to this study. First, we included patients with ALS within 3 years after receiving their diagnosis, as a longer disease duration of ALS would have led to a larger recall bias for the responses. Therefore, we reanalyzed only subjects who have been diagnosed within 1 year before the onset of ALS using a small number of cases (n = 39), but the results were similar to those derived from the whole data set (data not shown). In addition, there might be a survivor bias involved in collecting the data. In this study, deceased subjects were included, although the number of those was small (n = 25). To assess the scope of this potential problem, therefore, we repeated the analyses with the exclusion of deceased subjects, and the results were unaltered. Accordingly, the effects of survivor bias, if any, seem to be small.

Second, in this study information was provided by proxy respondents in approximately two thirds of the cases. As previously reported, however, proxy respondents provided acceptable information for smoking [28], episode of hypertension [28], and dietary information [29]. Moreover, the associations were unaltered after the data were reanalyzed with the exclusion of data obtained from proxy respondents (data not shown).

Finally, our questionnaire included questions on habits from 3 years before recruitment into the study. This is why we examine the causality between dietary intake of fruits and vegetables before the onset of ALS and the risk of ALS. Accordingly, it is possible that change in dietary habits might have occurred after initiation of ALS development. Therefore, we asked all of the cases whether or not they changed their dietary habits between 1 and 3 years prior to disease onset in the questionnaire; we confirmed that they had no significant change in dietary habits during the target duration. To avoid these problems, we are now planning a relatively large population-based prospective study.

In summary, we confirmed that higher intake of food rich in antioxidants such as fruits and vegetables is independently associated with a lower risk of ALS. Further prospective studies with more detailed information are needed to draw a conclusion as to the question of whether fruit and vegetables confer protection against ALS in Japan.

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ELSEVIER

Lifestyle Factors and Risk of Amyotrophic Lateral Sclerosis: A Case-Control Study in Japan

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PURPOSE: We examined the associations between lifestyle factors and the risk of amyotrophic lateral sclerosis (ALS) using a case-control study in Aichi Prefecture, Japan.
METHODS: The study comprised 183 ALS patients diagnosed by the El Escorial World Federation of Neurology criteria as well as 366 gender- and age-matched controls randomly selected from the general population with the use of the basic register of residents. Detailed information on lifestyle factors was obtained through a mailed self-administered questionnaire. The strength of association between ALS and a potential risk factor was assessed by calculating odds ratios (ORs) and 95% confidence intervals (CIs).
RESULTS: Vigorous physical activity, self reported stress, a type A behavior pattern, and less frequent intakes of green-yellow vegetables were significantly associated with increased risk of ALS, whereas smoking and drinking habits were not. The greatest effect on risk for ALS was posed by the combination of a type A behavior pattern and less frequent intakes of green-yellow vegetables (adjusted OR, 11.2; 95% CI, 3.8 to 33.0).
CONCLUSION: These data suggested that imbalances between excessive productions of oxidants as patient-specific factors and a diminished or missing antioxidant defense system in motor neurons may increase the risk of ALS.
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KEY WORDS: Amyotrophic Lateral Sclerosis, Epidemiology, Case-Control Study Personality, Oxidants, Antioxidants

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease of unclear etiology involving spinal cord motor neurons, leading to atrophy of the skeletal muscles, paralysis, and rapidly progressive death. ALS is considered to be a multifactorial disease in which complex environmental and genetic factors interact. Several experimental studies have proposed that oxidative stress contributes to the pathogenesis of ALS (1–3). Most studies have focused on vigorous physical activity (4–6), skeletal fracture (7), and exposure to heavy metals at work (8–10). According to Brooks (11), no risk factors, other than prolonged exposure at work or home to the agricultural chemicals in pesticides and herbicides, have been identified by epidemiologic studies. Recently, a few studies that have investigated lifestyle factors such as smoking (12, 13) and alcohol consumption (13) yielded inconclusive results.

Most prior studies have tested oxidative stress paradigms in mitochondria either through chemical inhibition of specific components of the respiratory chain or by adding an exogenous insult such as hydrogen peroxide or Paraquat to directly damage mitochondria. According to Hinerfeld et al. (14), antioxidant treatment enabled a reduction in the loss of neurons. Accordingly, it is quite likely that oxidative stress to brain nerves (e.g., cigarette smoking and vigorous physical activity) and antioxidant defense systems (e.g, copper-zinc superoxide dismutase [SOD1]) may be synergistically, rather than independently, associated with the development of ALS. However, to our knowledge, few studies have examined the joint effects of oxidative stress and antioxidant defense systems on the risk of ALS.

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Selected Abbreviations and Acronyms

ALS = amyotrophic lateral sclerosis
MET = metabolic rate
OR = odds ratio
CI = confidence interval

Therefore, using relatively large numbers of patients in Japan, we conducted a case-control study to explore the relationship between lifestyle factors related to oxidative stress, such as smoking, and antioxidant defense systems, such as higher intakes of antioxidant-rich food.

SUBJECTS AND METHODS

Study Populations

All case subjects were definite or probable ALS, who met the El Escorial World Federation of Neurology criteria for ALS that is widely used as the standard diagnostic criteria (15), aged 18 to 81 years with a disease duration of 3 years or less within the study period from 2000 to 2005 in six medical centers playing a major role in the diagnosis and treatment of ALS in the Tokai area.

Of the 274 ALS patients, 214 (75.3%) eligible cases were enrolled, 183 of whom completed the entire questionnaire. All cases of progressive bulbar palsy were included in this study, but those with familial progressive muscular atrophy were not. There was no evidence of coexisting Parkinson disease or related disorders, including multisystem atrophy.

The present study was approved by the institutional review board of the Aichi Prefectural College of Nursing & Health.

We set up two community controls that matched to each case for age (± 2 years) and gender. Therefore we selected quadruple controls to match each case, collecting more controls by a proportional simple random sampling with stratification by sex and age groups from among the general population in the same district as our cases based on the electoral register. Among a total of 732 eligible controls contacted, 550 (75.2%) were enrolled in this study and 430 completed the entire questionnaire. No significant difference in the gender and age distribution was observed between responders and nonresponders.

Data Collection

A structured self-administered questionnaire specifically designed for this case-control study was distributed and collected by mail from both patients and controls. We asked patients to recall their lifestyle within the 3 years before the onset of ALS and, for controls, within the 3 years before the interview. To facilitate the recall of lifestyles, including dietary habits, we inserted into the questionnaire some questions about their familial and social backgrounds at that time.

The questionnaire yielded information on demographic characteristics (gender, age, height, and present and past weight), medical history, physical activity, lifestyle factors (e.g., moking and drinking habits, frequency of green-yellow vegetable intake), and psychological status (e.g., self-reported stress, type A behavior patterns). Among those factors, we used physical activity, smoking and drinking habits, self-reported stress, type A behavior patterns as oxidants, and frequency of green-yellow vegetable intake as antioxidants.

Vigorous physical activity was defined as any such activity requiring 6 metabolic equivalents or greater (a 6-fold or greater increase above the resting metabolic rate), for example, jogging, running, bicycling, lap swimming, and playing tennis more than three times per week in one's leisure time. Type A behavior patterns were assessed by a 10-item scale specifically designed by Maeda (16) for Japanese persons. These were dichotomized into type A (≥ 17 points) and non–type A (<17) according to the criteria of Maeda. Participants were asked to report their stress intensity as "never/hardly" or, if stressed, "light," "moderate," or "high". Subjects responding "moderate" or "high" were considered "high," whereas those who responded "never/hardly" or "light" were considered "low". Smoking status was classified as current smokers or nonsmokers (including ex-smokers). Drinking status was classified as current drinkers or nondrinkers (including ex-drinkers). Subjects given the following five response options were asked intake frequencies of green-yellow vegetables: "never/seldom," "less than once a week," "1–2 times per week," "3–4 times per week," and "almost everyday," with the frequency 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.").

Statistical Analyses

Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated by multiple conditional logistic regression analysis. Tests for trend in logistic regression analysis were performed using exposure variables and treating the scored variables as continuous. Two-sided p values less than 0.05 were considered statistically significant. Statistical analyses were conducted using the Statistical Package for the Social Sciences version 14.0 (SPSS Japan, Inc,).

RESULTS

Table 1 shows that no significant difference in mean age was found between patients and controls.

As shown in Table 2, an increased risk of ALS was significantly associated with vigorous physical activity (OR = 2.0 for "heavy" compared with "not heavy"; 95% CI, 1.0 to 4.0), self reported stress (adjusted OR = 1.8; 95% CI, 1.3 to 2.7), and type A behavior pattern (adjusted OR = 2.9; 95% CI, 1.9 to 4.5), and less frequent intake of green vegetables (adjusted OR = 2.5; 95% CI, 1.7 to 3.7). No significant association with increased risk was found for either smoking or drinking habits.

Among the variables studied, the OR was highest for the association of type A behavior with oxidants; similarly, the OR was highest for the association of green-yellow vegetables with antioxidants. Accordingly, we selected type A behavior and green-yellow vegetable consumption as markers, respectively, for oxidants and antioxidants, in order to examine their joint effect on ALS risk.

As shown in Table 3, the greatest effect on risk for ALS was posed by the combination of a type A behavior pattern and a less frequent intake of green-yellow vegetables (adjusted OR = 11.2; 95% CI, 3.8 to 33.0), a less frequent intake of green vegetables without a type A behavior pattern posed a slightly but significantly elevated ALS risk.

DISCUSSION

In this case-control study, we found that patient-specific factors such as type A behavior patterns and less frequent intakes of green-yellow vegetables, when combined, may be more strongly associated with an increased risk of ALS, compared with environment specific factors, such as smoking habit.

We found that the joint effects of a type A behavior pattern and less frequent intake of green-yellow vegetables was strongly associated with an increased risk of ALS. As far as we know, no epidemiological information is available about the joint effect of oxidants and antioxidants on the risk of ALS. This is the first, to our knowledge, epidemiological study to demonstrate the joint effect of oxidants and antioxidants on the risk of ALS.

TABLE 1. Comparison of selected characteristics of cases and controls

	Cases (n = 153)	Controls (n 306)	
	% or Mean	% or Mean	*p* Value
Sex			
Men	60.3	60.3	
Women	39.7	39.7	NS
Age group (yr)			
≤49	32.6	33.3	
50–59	36.4	34.8	
≥60	31.0	31.9	
Mean age (± SD)	63.7 ± 9.2	63.4 ± 10.6	NS
BMI	22.2 ± 0.2	23.3 ± 0.3	<0.05

NS = not statistically significant; SD = standard deviation; BMI = body mass index.

TABLE 2. Odds ratios and 95% confidence intervals for ALS according to lifestyle factors

	Cases (n = 153)	Controls (n = 306)	Adjusted OR*	95% CI
Bone fracture				
No	81.3	84.9	1.0	
Yes	19.6	15.1	1.3	0.9–2.1
Vigorous physical activity				
No	88.4	93.8	1.0	
Yes	11.6	6.2	2.0	1.0–4.0
Hate to lose				
No	37.0	43.4	1.0	
Yes	63.3	56.6	1.3	0.9–1.9
Self-reported stress				
Low	38.1	52.6	1.0	
High	61.9	47.4	2.9	1.3–2.7
Behavior pattern				
Non–type A	55.8	80.4	1.0	
Type A	44.2	19.6	2.9	1.9–4.5
Lack of body pliableness				
No	37.4	24.8	1.0	
Yes	13.7	24.5	1.1	0.8–1.6
Smoking				
Nonsmoker	51.9	51.5	1.0	
Current smoker	48.1	48.5	1.0	0.6–1.3
Drinking				
Nondrinker	30.9	32.0	1.0	
Current drinker	69.1	68.0	1.1	0.7–1.5
Intake of green-yellow vegetables				
Frequent	49.2	29.4	1.0	
Less frequent	50.8	70.6	2.5	1.7–3.7

ALS = amyotrophic lateral sclerosis; OR = odds ratio; CI = confidence interval.
*Odds ratios were calculated for each lifestyle variables adjusted for age, sex, all above variables other than target variable.

A methodological issue was analyzed for combined sporadic and familial ALS. In this study, we had a small proportion of subjects with positive familial ALS (1.9%). These findings suggest that the sporadic and familial forms of ALS are clinically and pathologically indistinguishable. Moreover, we used self-administered questionnaires to obtain information retrospectively. Therefore, the reliability of self-administered questionnaires was assessed by using the test-retest method over a 2-week period for 50 persons randomly selected from among community controls. Kappa values ranged from a low of 0.52 for general stress to a high of 0.86 for smoking habit, suggesting that the measures in this study might be considered adequately reliable.

TABLE 3. Odds ratio (95% CI) for ALS by type A behavior and intake frequency of green-yellow vegetables

Type A behavior	Intake of green-yellow vegetables	Cases (n = 153)	Controls (n = 306)	Adjusted* OR (95% CI)
No	Frequent	46.4	65.9	1.0 (reference)
No	Less frequent	41.4	28.1	1.9 (1.3–3.4)
Yes	Frequent	4.5	4.7	1.5 (0.6–3.5)
Yes	Less frequent	7.7	1.2	11.2 (3.8–33.0)

CI = confidence interval; ALS = amyotrophic lateral sclerosis; OR = odds ratio.

As for psychological factors, McDonald et al. (17) reported that psychological status is strongly related to a greater risk of mortality in ALS. However, few studies have examined the relationship of psychological factors to the risk of ALS, though several have demonstrated that psychological stress is associated with increased oxidative stress (15, 18). In the present study, we not only found high self-reported stress, but also discovered that type A behavior patterns identified as stress-producing behavior (19) were significantly associated with an increased risk of ALS, suggesting that personality factors may promote the disease. In addition, we found that the magnitude of the risk from type A behavior patterns was highest among the other lifestyle factors used as sources of oxidative stress. Watkins et al. (20) reported that a type A behavior pattern is related to emotional distress and to more keenly perceived life stress. Accordingly, these findings suggest that a type A behavior pattern may be associated with an increased risk of ALS indirectly, but not directly psychological stress.

We observed that the less frequent intakes of green-vegetables was significantly associated with an increased risk of ALS; it is also well known that increasing the intake of fruit and vegetables may protect against oxidative stress by acting as a free-radical scavenger system (21, 22). However, there is little available information on the relationship between one's daily intake of vegetables and the risk of ALS. Our results suggested that high-frequency green-yellow vegetable intake may be protective against the development of ALS.

A few studies have examined the relationship between cigarette smoking and the risk of ALS (12, 13, 23), but the relationship was found to be inconsistent. However, we could find no significant relationship at all between cigarette smoking and the risk of ALS.

These findings suggest that type A behavior patterns and less frequent intakes of green-yellow vegetables considered as patient-specific (endogenous) factors, when combined, may be more strongly associated with an increased risk of ALS, compared with smoking habit considered as environment-specific (exogenous) factors.

To our knowledge, few studies on the risk of ALS have simultaneously compared the effect of patient-specific (endogenous) factors such as type A behavior patterns with environment-specific (exogenous) factors such as smoking. This is the first epidemiological study to demonstrate that patient-specific (endogenous) factors are more likely than environment-specific (exogenous) factors to be strongly associated with the risk of ALS.

Moreover, we found that the ALS risk was greatest in subjects only who had both type A behavior pattern as oxidants and a lesser frequency of green-vegetable intake as antioxidants.

Oxidative stress and mitochondrial dysfunction have been linked to neurodegenerative disorders such as Parkinson's and Alzheimer's disease (24). Hinerfeld et al. (14) also reported a striking pattern of neuronal cell death as a result of mitochondrial oxidative stress in SOD2 null mice. Moreover, Shiba and Shimamoto (25) found that endogenous oxidative stress under treatment with a combination of 3-amino-1,2,4-triazole and mercaptosuccinic acid induced cell death that resembles apoptosis; they also found that endogenous oxidative stress was directly related to the cytochrome P450 enzyme system. Accordingly, these findings would provide an explanation for our result that endogenous oxidants rather than exogenous ones may be more strongly associated with an increased risk of ALS.

Hinerfeld et al. (14) reported that antioxidant treatment brought about a reduction in the loss of neurons. In addition, Niebroj-Dobosz et al. (26) proposed the necessity of an antioxidative neuroprotective strategy in ALS, since the disease results from oxidative damage to spinal cord proteins. These findings suggest that the synergistic effect of oxidation and antioxidation systems may be associated with the development of ALS, rather than acting independently. However, very little is known about the relationship between the oxidation/antioxidation balance and the risk of ALS. Our results suggested that the excessive endogenous oxidants and a decrease in or lack of an antioxidant system may, when combined, increase the risk of ALS. As far as we know, this is the first case-control study of ALS that demonstrated an association between the oxidants/antioxidants imbalance and the risk of ALS.

There are several limitations to this study. First, the information in this study was provided by proxy respondents in approximately half of the cases. Consequently, the possible effect of potential bias by proxy respondents has to be seriously considered. Some studies have previously reported that proxy respondents provided reliable information for smoking (27), episodes of hypertension (27), and some kinds of dietary information (28). Moreover, those associations were unaltered after the data were reanalyzed by the exclusion of data obtained from proxy respondents (data not shown).

Second, our questionnaire requested information on several points 3 years before the onset of ALS. This was done because we sought to examine the causality between lifestyle factors before the onset of ALS and any subsequent of risk. Accordingly, the possibility was not discounted that lifestyle exposures might have changed after the initiation of

ALS development. Therefore we have confirmed that none of the respondents exhibited any significant change in their lifestyle between a period of 3 and 10 years prior to disease onset. To avoid these problems, we are now planning a relatively larger population-based prospective study.

Third, we used self-administered questionnaires to collect information on both cases and controls. The above authors had demonstrated no significant difference in the responses to questions related to lifestyle factors between self- and interviewer-administered questionnaires (29). The relationship between vigorous physical activity and the risk of ALS in this study was similar to the one in previous studies conducted using interviewer-administered questionnaires (4–6). These findings suggest that the effect of our collection method on the responses may well be a minimal one.

Finally, we assessed nutritional status using the frequency of green-yellow vegetable intake rather than the amounts of antioxidant intake. A few studies have demonstrated that green-yellow vegetables and fruits enhanced the plasma antioxidant status (23, 30, 31). Drewnowski and Hann (32) reported that mean frequencies of food consumption were a significant predictor of dietary outcomes. Further studies will be needed to confirm the relationship between the amount of antioxidant intakes and the development of ALS.

In summary, the present study clearly suggested that imbalances between the excessive production of oxidants originating from patient-specific factors and the decrease in or lack of an antioxidant system in motor neurons may increase the risk of ALS.

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