We conducted a comparative analysis for the differences of these parameters in the elderly groups depending upon several attributes: illness (inpatients or outpatients), residence (institution or home), and sex (male or female).

Materials and methods

Seventy-three male and 71 female geriatric inpatients (aged ≥ 65 years) who were admitted to Nagoya University Hospital in the year 1991–1995 were enrolled in the present study (inpatient group). The major causes of admission were cerebrovascular disease (17.4%), infections (12.5%), and malignancy (12.5%). Eighty-eight male and 149 female geriatric outpatients who were treated in the hospital were also included (outpatient group).

Two hundred and 96 nursing home residents were asked to complete a questionnaire that included items on amount of food intake, activities of daily living (ADL) including ability to walk for 10 min without aid, medications, past history of illness, and participation in club activities. A total of 68 male and 78 female residents were selected according to the selection criteria; namely that they were independent, ambulatory, consumed more than 80% of served meals (total energy intake: 358.5–370.5 KJ), and had no serious diseases requiring specific treatment. Informed consent was obtained at the end of 1994 from these participants to take part in the present study (nursing home group).

In addition, examinees of mass health check-ups who visited Aichi General Health Center in 1992 were included and divided into two groups: an elderly group (aged ≥ 65 years, 68 male and 52 female subjects; health check-up group) and a younger group (aged 30–64 years, 417 male and 95 female subjects; control group).

Most of these examinees were healthy and living in the community. The demographic characteristics of a total of 10 subgroups are given in Table 1. The mean age of each group did not differ with statistical significance, except for a significantly lower mean age in the elderly health check-up group compared to other female groups.

Fasting blood samples were drawn from the antecubital vein in the morning in inpatients, nursing home residents, and examinees of mass health check-ups. In outpatients the blood sampling was also done in the morning but these samples were not always fasting ones. The blood was drawn with examinees in the sitting position, except for inpatients whose blood was drawn in the supine position on the second day of admission.

In the groups of inpatients and outpatients and residents, hemoglobin (Hb) and white blood cell (WBC)

Table 1 Numbers and age characteristics of each group

	Inpatients		Outpatients		Nursing home residents		Health check-up group		Control	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
n	73	71	88	149	68	78	68	52	417	95
Age (mean ± SD)	77.4 ± 6.7	76.5 ± 7.1	75.7 ± 6.6	75.2 ± 6.6	75.4 ± 6.3	76.5 ± 6.0	75.4 ± 6.3	72.3 ± 2.7	51.3 ± 6.6	51.4 ± 6.7

SD, standard deviation.

levels were determined by hemocytometer. Serum albumin (Alb), calcium (Ca), sodium (Na), creatinine (Cr), and total cholesterol (TC) were measured by multiple analyzer (735-Toshiba™), and in the groups of residents of nursing home and health check-ups we transformed the observed data according to the regression formula (in the case of TC, $Y = 1.0315X + 0.12887$, $r = 0.9974$). These regression formulas were obtained using several samples. Albumin-corrected calcium was calculated according to the formula,[1] with minor modification of Payne's formula[2] as follows: (corrected calcium (mEq/L) = measured Ca (mEq/L) − 0.355 × Alb (g/dL) + 1.6).

Statistical methods

The values of Hb, Alb, and Cr exhibited a Gaussian distribution, while those of WBC, Ca, corrected Ca, Na and TC did not. Statistical analysis was made using ANOVA after appropriate transformation of these values. Transformations for these parameters were as follows: WBC (logx), Ca, corrected Ca ($x^{1/3}$), Na ($x^{3/2}$), and TC ($x^{1/2}$). For each subgroup the arithmetic mean and standard deviation were determined, and are shown in the figures. As a post hoc test, Scheffe's method was employed to examine the statistical significance of the differences among the male and female subgroups; the significance level was set at $P < 0.05$. Students' *t*-test was also used for comparison between the sexes in each group.

Results

As shown in Fig. 1, the mean Hb concentration was lowest in inpatients, with a stepwise rise from male inpatients to young controls. In women there was a smaller stepwise rise from inpatients to nursing home residents but no statistical differences in Hb concentration were seen among nursing home residents, the health check-up group, and young controls. The mean Hb concentrations were similar in these groups. The gender differences (male > female) were significant in all pairs of groups (Table 2).

The WBC count was significantly higher in inpatients of both sexes than in controls (Fig. 2).

Serum albumin had markedly lower values in inpatients of both sexes and higher values in male controls (Fig. 3). As seen with Hb concentrations, no differences in Alb levels were found among female nursing home residents, the health check-up group, and controls.

Serum levels of Ca and Na were determined in inpatients, outpatients and nursing home residents. Significantly lower values of serum Ca were found in inpatients than in outpatients and the nursing home group. Correction of Ca concentration with Alb resulted in similar values in the three groups (Fig. 4).

Significantly lower values of Na were found in inpatients than in outpatients and the nursing home group, suggesting a higher prevalence of hyponatremia among elderly inpatients (Fig. 5).

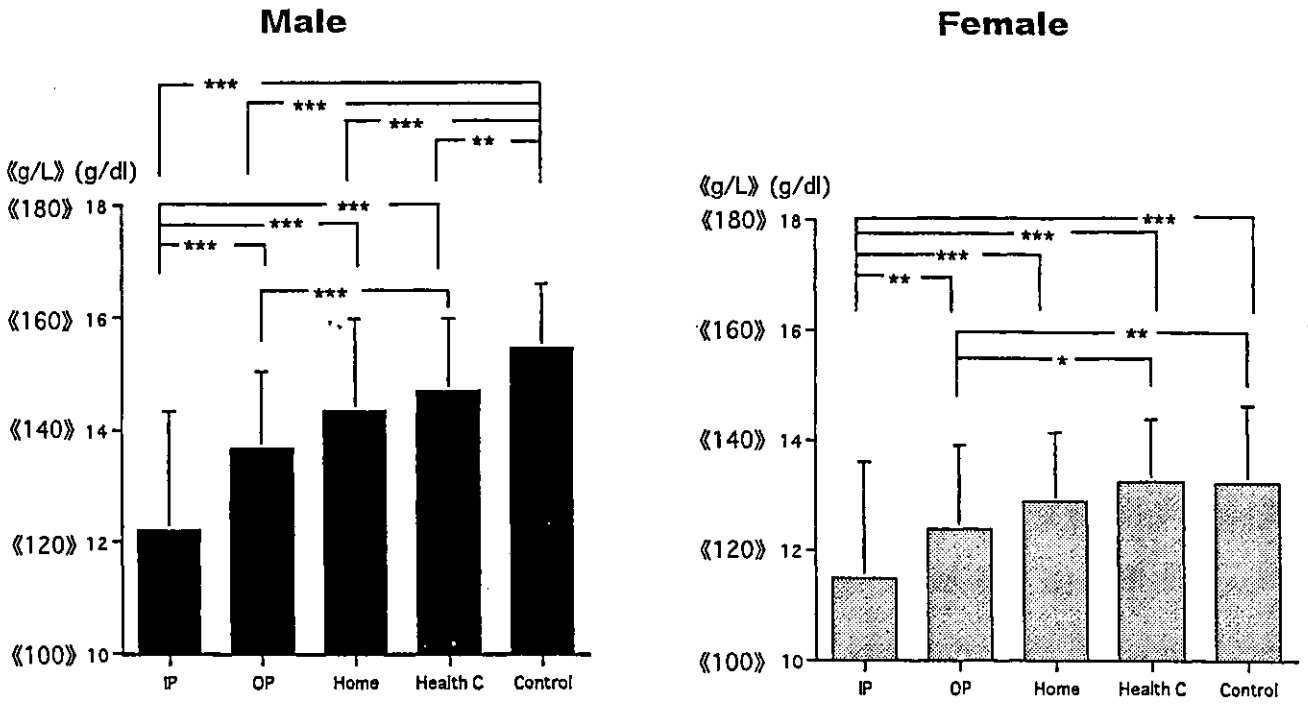

Figure 1 Mean hemoglobin concentration. IP, inpatients; OP, outpatients; Home, nursing home residents; Health C, health check-up group. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 2 Gender differences between each pair of groups

	Hb	WBC	Alb	Ca	cor-Ca	Na	Cr	TC
Inpatients	*	NS	NS	NS	NS	NS	NS	NS
Outpatients	***	*	*	***	NS	NS	***	***
Nursing home residents	***	**	NS	S	NS	***	***	**
Health check-up group	***	NS	**	***	***			
Control	***	***	***	***	NS			

Hb, serum hemoglobin concentration; WBC, white blood cell count; Alb, serum albumin concentration; Ca, serum calcium concentration; cor-Ca, albumin corrected calcium concentration; Na, serum sodium concentration; Cr, serum creatinine concentration; TC, serum total cholesterol concentration; NS, not significant.
$*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

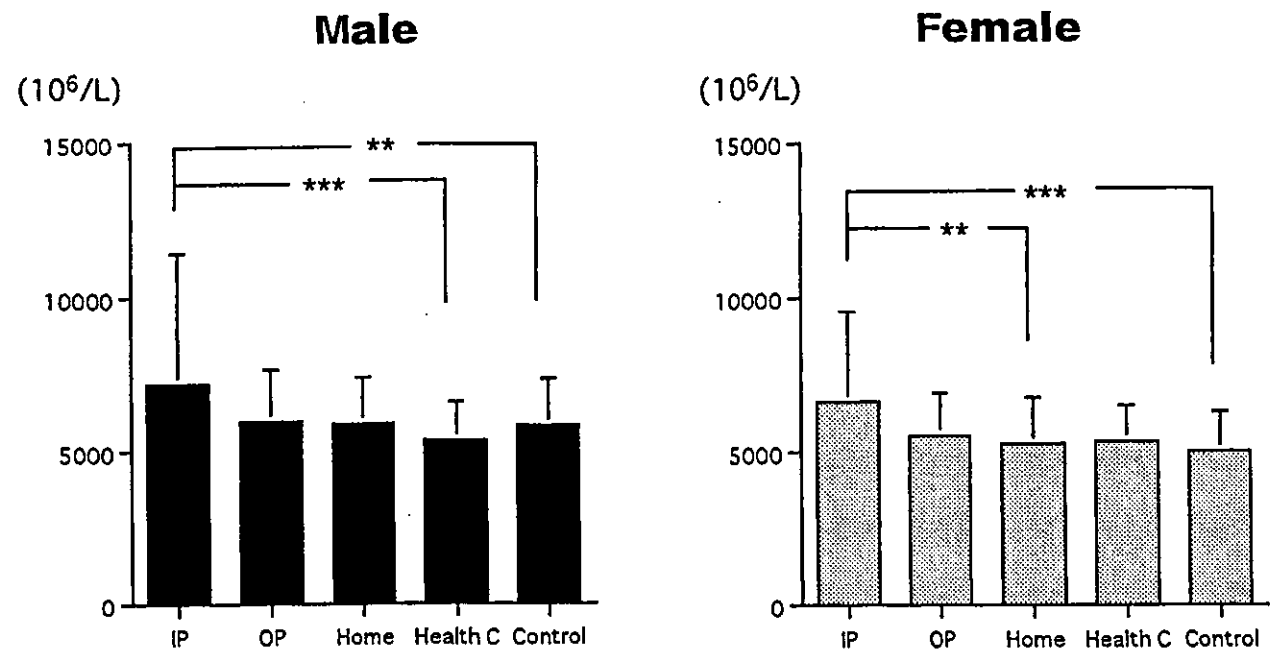

Figure 2 Mean white blood cell count. IP, inpatients; OP, outpatients; Home, nursing home residents; Health C, health check-up group. $**P < 0.01$; $***P < 0.001$.

There was a tendency for a stepwise decrease in Cr concentration from inpatients to young controls (Fig. 6). Remarkable gender differences (male > female) were found in each pair of groups other than inpatients (Table 2). These distinct gender differences may support the establishment of a separate reference range of serum Cr for both sexes in the elderly as well as younger adults.

The mean levels of serum TC were high in elderly women other than inpatients, exceeding 220 mg/dL in outpatients and the health check-up group. Female outpatients and the health check-up group had significantly higher values than inpatients, whereas in men significantly lower levels in inpatients were observed compared to the health check-up group (Fig. 7). In the elderly groups other than inpatients, women had significantly higher TC values than men.

In summary, there were significantly lower levels of Hb, Alb, Ca, Na, TC, and in contrast higher levels of WBC and Cr, in inpatients than in other groups in both sexes. Outpatients were found to have lower levels of Hb than the health check-up group and controls, and higher levels of Cr in men compared to controls. The gender differences in each pair of groups are summarized in Table 2. There were gender differences in Hb, Cr (male > female), and TC concentrations (male < female; Table 2). Among women there was no difference in the levels of any parameters between nursing home residents, health check-up examinees, and controls.

Discussion

The Hb concentrations were reported to decrease with age in the community-dwelling male elderly, while no

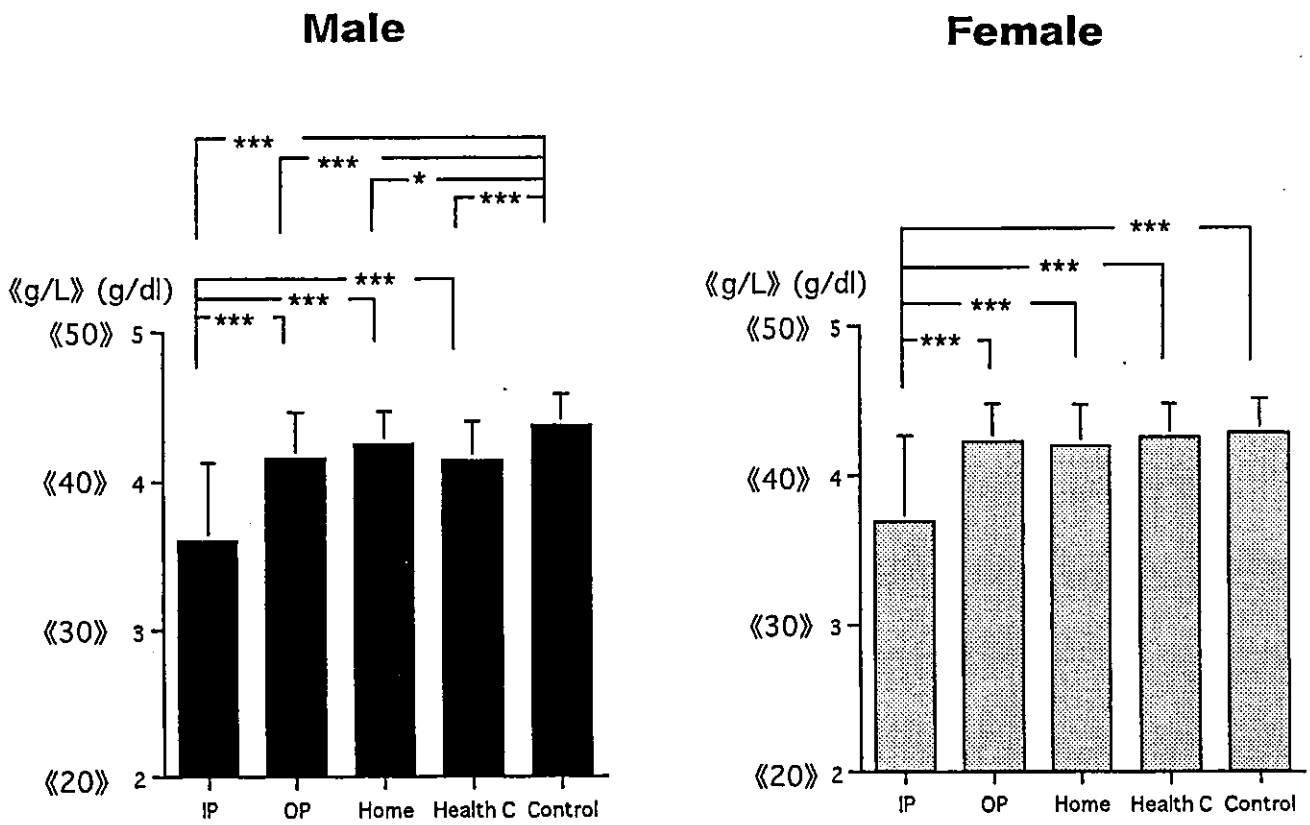

Figure 3 Mean serum albumin concentration. IP, inpatients; OP, outpatients; Home, nursing home residents; Health C, health check-up group. $^{*}P < 0.05$; $^{***}P < 0.001$.

significant decrease was found in women until the age of 80.[3] In the present study, among women no difference was found in Hb concentration between nursing home residents, health check-up examinees, and controls. It is likely that Hb value does not change with age in healthy women.

White blood cell count was reported to decrease with age.[4-6] In the present study, although WBC was high in inpatients, there was no difference in the other four groups, suggesting the existence of infective or inflammatory disease at the time of hospitalization.

Serum albumin is known to be a good indicator of nutritional status.[7-9] In the present study the Alb levels in male outpatients, nursing home residents and health check-up examinees groups were clearly lower than in the control group. In a recent longitudinal study in Japan, the Koganei Study, Shiabata reported that Alb levels declined significantly with age, irrespective of the presence or absence of disease.[10] In the present study Alb levels were significantly lower in the inpatient group than in all of the other groups for both men and women, which would seem to be strongly affected by the diseases; the various diseases that were the major cause of admission may account for the hypoalbuminemia at the time of hospitalization. In women, the lack of significant differences among the control group and the outpatients, nursing home residents, and health check-up examinees groups indicates that the levels of Alb as well as Hb can be maintained in the aged.

Because 45% of total Ca is bound to Alb, serum Ca levels decline in parallel with Alb. However, it was emphasized that ionized Ca, which has a key role in biological activity,[2] is maintained at normal levels.[11,12]

Albumin-corrected calcium has been calculated according to Payne's formula.[2] However, the boundary Alb levels were set at 4.0 g/dL, which was lower than the average levels in our subjects except for inpatient groups (Fig. 3), so applying this formula directly to our study might underestimate Ca levels in many subjects with Alb levels > 4.0 g/dL. The modified formula of Payne's correction[1] eliminated the differences resulting from Alb levels, indicating the need for Alb correction in evaluating Ca concentration in the elderly.

The significantly lower Na levels in both male and female inpatients are thought to reflect the prevalence of hyponatremia at the time of hospitalization. This may be the frequent comorbid condition in terms of electrolyte balance among the hospitalized elderly.

The renal glomerular filtration rate declines with age; therefore, creatinine clearance (Ccr) also declines.[13] Our data show no elevation in Cr in the elderly. Because of decreased muscle mass in the elderly, it is apparent that

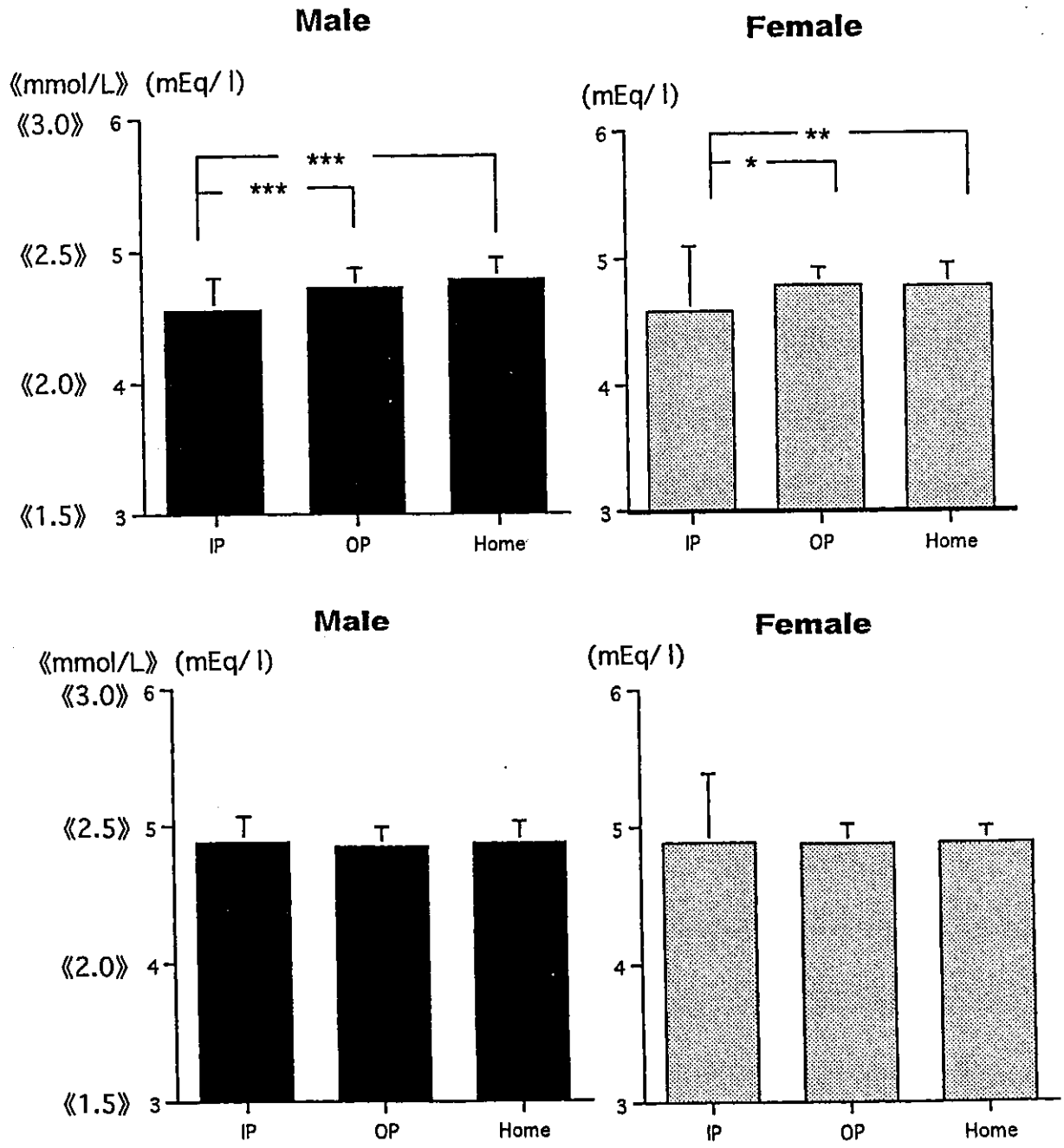

Figure 4 Effects of albumin concentration in the mean serum calcium concentration. Upper, measured calcium concentration; lower, albumin-corrected calcium concentration. IP, inpatients; OP, outpatients; Home, nursing home residents. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

the Cr level does not rise even if Ccr declines.[13] A clear gender difference was also seen between elderly men and women (men > women). The lower Cr levels in women than in men were thought to be due to lower muscle mass. The Cr level was higher in hospitalized patients, most likely attributable to the impaired renal function that is a common complication at the time of hospitalization.

The high serum cholesterol levels found in the present study suggest a high prevalence of hypercholesterolemia in the elderly, especially in women. Recent study has shown that the levels of serum cholesterol are kept high until 70 years in Japan.[14] Unlike the elderly living in the community, the inpatient group, who were in the acute phase of disease, had significantly lower values than other groups. This is probably due to the influence of the disease for which they had to be hospitalized, or indicates malnutrition accompanying the disease.

The nursing home in the present study was established before the introduction of the long-term care

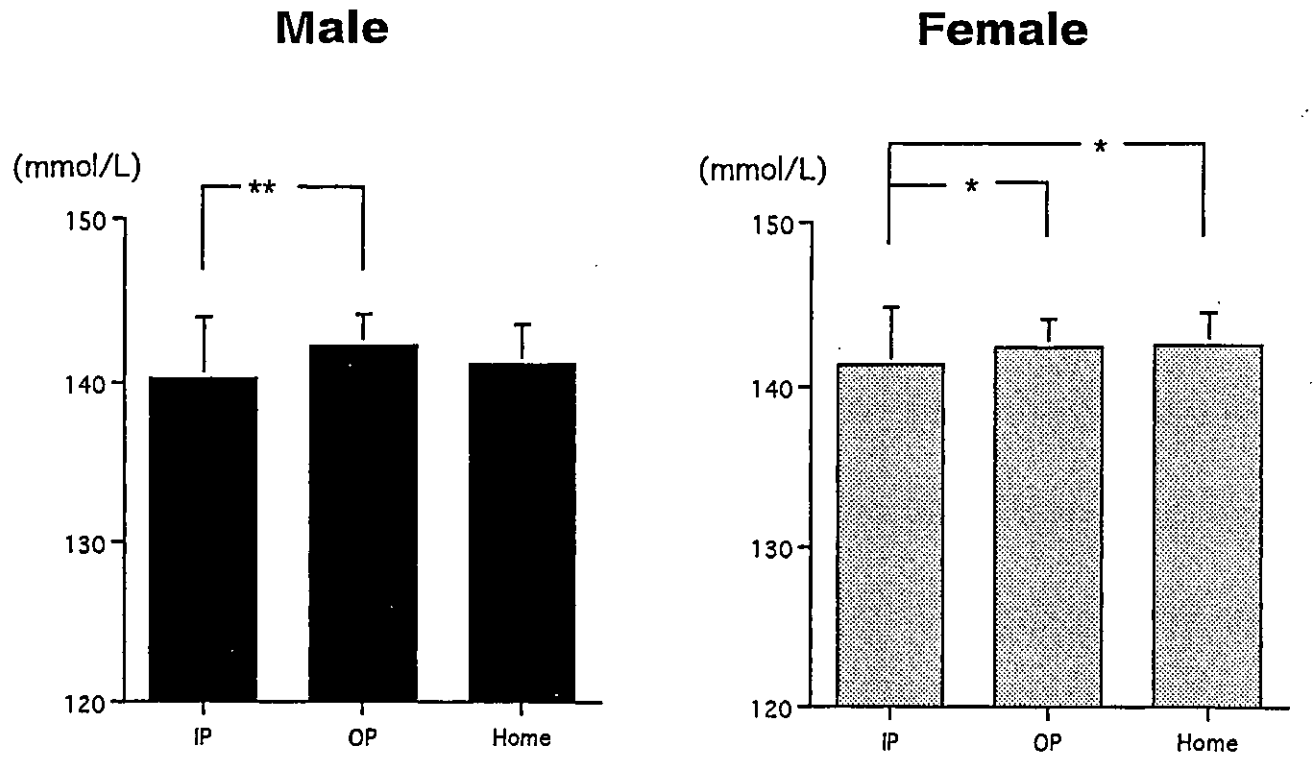

Figure 5 Mean serum sodium (Na) concentration. IP, inpatients; OP, outpatients; Home, nursing home residents. *$P < 0.05$; **$P < 0.01$.

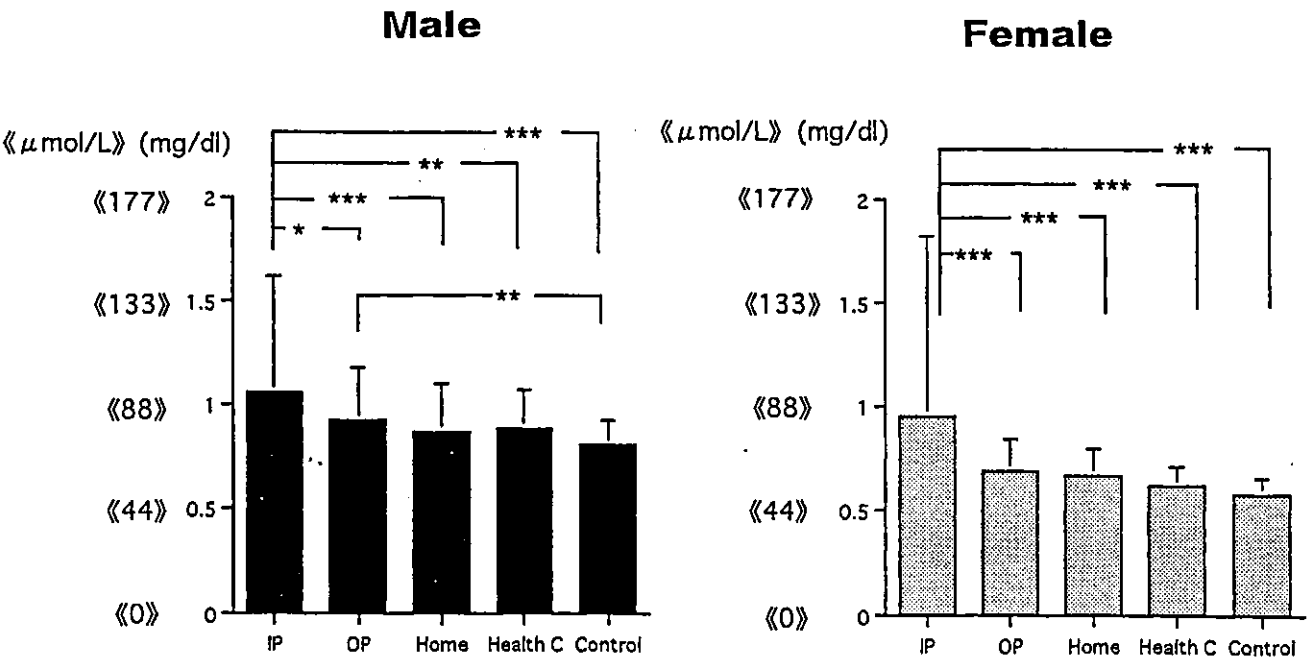

Figure 6 Mean serum creatinine (Cr) concentration. IP, inpatients; OP, outpatients; Home, nursing home residents; Health C, health-check-up group. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

insurance system in Japan, where relatively healthy elderly lived with a fair degree of independence, and energy intake was controlled at 358.5–370.5 J per day. It is possible that the level of independence including ADL and the state of nutrition and morbidity differ from those in special nursing homes today.

In elderly women the prevalence of hyperlipidemia is higher than that in younger women.[15] Differing from

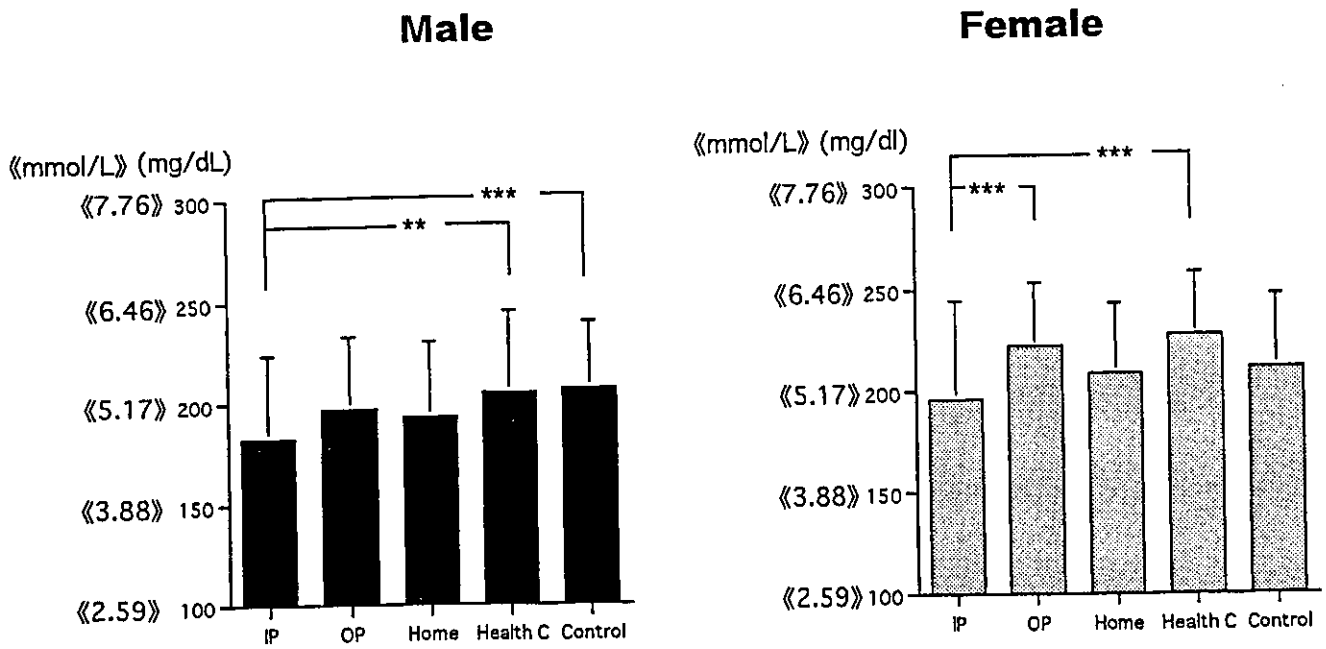

Figure 7 Mean serum total cholesterol concentration. IP, inpatients; OP, outpatients; Home, nursing home residents; Health C, health check-up group. $^{**}P < 0.01$; $^{***}P < 0.001$.

those in community dwelling elderly, the serum cholesterol levels in institution-dwelling elderly may be affected by the meals provided, which have relatively fixed calories.

In summary, the most noteworthy finding from the present study is that the Hb, Alb, and TC levels in relatively healthy women were not lower than those in the control group. This may be an important finding consistent with good nutrition.

The present study investigated the effects of the attributes of groups such as inpatients, outpatients, nursing home residents, and people undergoing health checks upon the differences in hematological or biochemical parameters. Our data indicate that major hematological or biochemical parameters are affected to a greater extent in inpatients. Morbidity from various diseases, a variety of comorbid conditions, or malnutrition may account for the different levels of these parameters in patients. Female nursing home residents and health check-up examinees have similar values to younger controls, which may be connected to the longevity of women.

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第45回日本老年医学会学術集会記録
〈市民公開在宅介護フォーラム：在宅介護を進めるために〉

1）在宅介護における予防医学～要介護度の悪化を防ぐ～

安藤富士子

〈要　約〉　在宅介護を推進するためには質と効率の高い介護の供給とともにADLの低下を防ぎ，新たに要介護状態となる虚弱老人を減少させる予防医学的な方法論が必要である．寝たきり老人の中で，疾患発症により直接に寝たきりになったものは約30%に過ぎないと言われている．二次的に「寝たきり」を引き起こす要因として重要なものに「廃用症候群」と「閉じこもり」がある．安静や不動によってもたらされる廃用症候群は筋力の低下や骨密度の減少，知的関心の低下，感染症などをきたし，さらにADLを悪化させる．生活に密着した日々のリハビリテーションが廃用症候群の予防には重要である．さらに前段階のADL悪化要因として最近，高齢者の「閉じこもり」が重用視されている．「閉じこもり」には，加齢や身体的要因のほか，尿失禁や転倒を怖れるための外出恐怖などの心理的要因，社会的役割・家庭内での役割の喪失といった社会的要因や環境要因が関連している．

脳血管障害や大腿骨頸部骨折など，身体的なADL低下要因を予防するとともに，高齢者の社会参加や知的関心を高めることや若い時期からの運動習慣が要介護高齢者を減少させるための予防医学的な方策として重要である．

Key words：寝たきり，閉じこもり，要介護，予防医学

（日老医誌　2004；41：61—64）

緒　言

在宅介護を推進するためには質と効率の高い介護の供給とともに，介護度の悪化を防ぎ，新たに要介護となる虚弱高齢者を減少させる予防医学的な方法論が必要である．介護を必要とする最たる状況はいわゆる「寝たきり」である．「寝たきり」の原因疾患として従来から脳血管障害，骨折，痴呆などがあげられているが，こういった疾患で直接「寝たきり」になるのは「寝たきり」の30%程度と考えられており，多くの「寝たきり」は慢性疾患や「閉じこもり」などによる心身の活動性の低下から二次的におこってくると考えられるようになってきた．

本講演では，在宅介護におけるADLの悪化の問題点について，特に「寝たきり」と「閉じこもり」に焦点を当てて検討し，在宅介護において介護度悪化を予防する方法論について論じ，さらに健常中高年者が心身ともに健康であり続けるための方策について言及した．

在宅介護とADL

65歳以上の高齢者において平成13年度までに介護保険の認定を受けた288万人のうち，約90%が介護サービスを受給しており，その70%が在宅で介護を受けている．在宅介護を推進するためには，「人（マンパワー）」と「場所（介護に適した居宅）」が必要であるが，それとともにADL（日常生活動作）の悪化を防ぐ方法論や本人や家族のQOLを考慮したゴールの設定が必要である．

いわゆるADLには入浴や身だしなみ，歩行やその他の移動方法，食事やコミュニケーションが含まれるが，このうち移動に関する能力は，障害の進行とともに，屋外から屋内へ，そして，室内，車いすへと可動範囲が狭くなっていき，最も重症な場合には寝たきり（bed-ridden）となる．

寝たきりの原因論とその予防

平成10年度国民生活基礎調査[1]によれば，寝たきりの原因は，脳血管障害(36.6%)，高齢による衰弱(13.5%)，骨折・転倒（11.8%），痴呆（9.0%）などである．

（1）脳血管障害

寝たきりの原因の約3分の1を占める脳血管障害は死亡原因の第3位である．高血圧症のコントロールにより，脳出血による死亡数は昭和45年以降激減したが，患者数は昭和62年の114万人から，平成11年の147万人まで3割近く増加している．患者数としては，国民病であ

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る高血圧症，糖尿病についで第3位であり，特に障害を有する疾患としては第1位となっている．

脳血管障害発症後の退院先を決定するADL要因として，排尿・排便の自立の重要性が報告されている[2]．すなわち排尿・排便が自立していれば，自宅での療養が可能となる頻度が有意に高く，逆に排尿・排便に介護が必要であると施設や病院に転院となる可能性が高くなる．食事や着替えなどの介護に比べて，排尿・排便の介護は，頻度が高く，時を選ばず，また着替え動作を伴うために肉体的負担も大きい．したがって排尿・排便の自立を存続させることが，在宅介護を継続させる上でも大きな鍵になると考えられる．

(2) 大腿骨頸部骨折

骨折の中で特に大腿骨頸部骨折が寝たきりの原因となる．宮田ら[3]によると平均年齢77.9歳の大腿骨頸部骨折患者40人を調査したところ，ADLが元のレベルまで戻るのは約30%であり，約半数ではADLが一段階低下しており，15%では歩行器や伝い歩きさえもできなくなった．歩行不可となった人の3分の2はもともと，屋内を歩行器，あるいは伝い歩きで移動していたADLの低い人たちであった．一方，受傷以前に屋外独歩が可能であった群からは一人も寝たきりが発生しなかった．このことから，大腿骨頸部骨折後のADLには受傷以前のADLが密接に関与していることが理解される．日頃，身体を実際に動かしている人ほど，骨折後の「寝たきり率」は低いのである．また，ADLは転びやすさとも関連しており，このことからも日頃身体を動かして，ADLを高く保つ努力が，大腿骨頸部骨折による寝たきりを防ぐと考えられる．

二次的寝たきりとその原因

脳血管障害や大腿骨頸部骨折などの発症後，直接寝たきりになる（図1-A）率は，寝たきり全体の約1/3程度にすぎないと報告されている[4]．寝たきりになる経過としてこれ以外にしばしば認められるのは①脳血管障害などで，一旦ADLがある程度まで下がり，その後，再発作や廃用症候群などでADLが段階的に低下するパターン（図1-B），②明らかな疾患の発症がないままに徐々にADLが低下するパターン（図1-C）などである．

(1) 廃用症候群

廃用症候群（disuse syndrome）とは心身を使わないことによって，その機能が衰えてしまうことであり，高齢者では数日の寝たきりで筋肉の萎縮や関節の拘縮が起こるばかりか，循環器系や呼吸器系などの生理機能の低下や起立性低血圧など自律神経失調もきたすため，ますます起きあがることが困難となり，それに伴って自立心も低下し，知的な関心も落ちていくことが多い．このような悪循環が寝たきりを2次的に作り出す．従って日頃，介護の中でADLを下げないために戦略として，特に廃用症候群の予防や運動，栄養，心の満足や知的関心に注意を払うべきである．

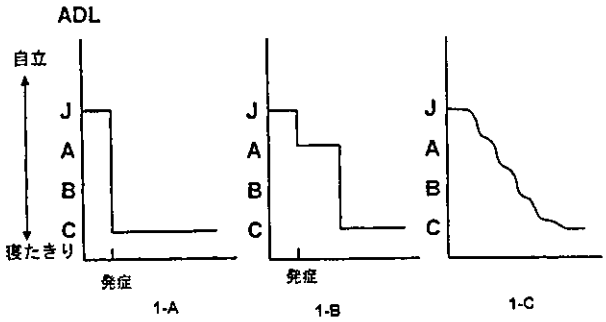

図1　高齢者が寝たきりとなる時間的経過（文献[1]から改変）
すべての寝たきり患者が疾患の発症により，直接寝たきりになるわけではない．
1-A．脳血管障害，大腿骨頸部骨折などの疾患の発症により，すぐに寝たきりになるパターン．
1-B．疾患の発症によりある程度ADLが低下した後，廃用症候群や再発で段階的にADLが低下するパターン
1-C．明確な疾患の発症がないまま，徐々にADLが低下するパターン
縦軸は「障害老人の日常生活自立度（寝たきり度）判定基準」[5]によるADLを，横軸は時間経過をそれぞれ模式的に示している．

具体的には，生活に即したリハビリ，すなわち患者の生活に直接役立つようなリハビリを一日の生活の中に取り入れて行う．車いすに座らせておく，というような見かけ上のADL向上や廊下を毎日何往復する，というような義務としてのリハビリは高齢者のQOLを改善しない．臥位になりがちな人では，自立座位の時間を延ばすことによって，背筋力，平衡感覚を鍛えるとともに，食事や会話を座ってできる喜びを味わってもらうことが大切である．ベッドサイドでの自立座位が確保されると，つかまり立ちから，少し身体をひねるだけで，ポータブルトイレに座ることが可能となり，前述した在宅介護に重要な排尿・排便のADLが確立される．こういった生活に即したリハビリのほうが実効的であり，患者や家族の達成感や，負担の軽減にも役立つ．また，毎日身体を動かすこと，特に健側の筋力を低下させないことも重要である．

(2) 閉じこもり

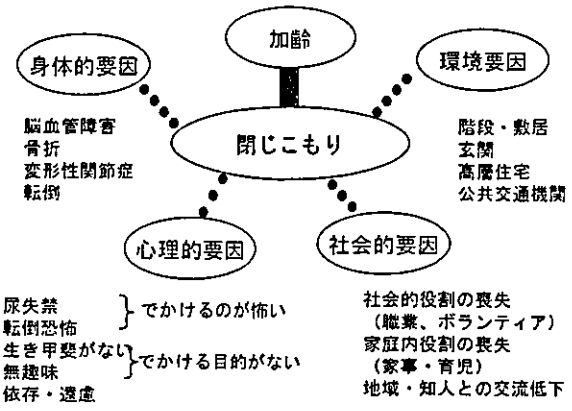

図2　閉じこもりの関連要因
加齢は不可逆要因であるが，その他の要因は予防や改善が可能と考えられる．

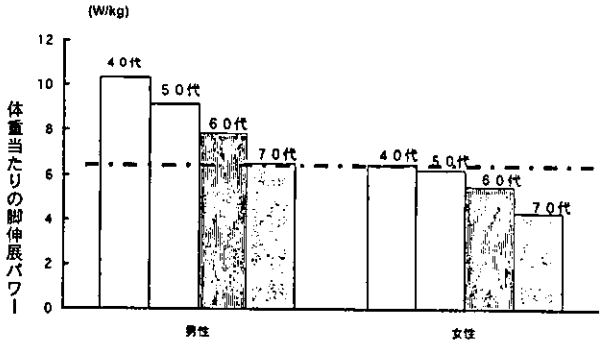

図3　脚瞬発力の加齢変化(「長寿医療研究センター・老化に関する長期縦断疫学調査」第一次調査結果より)
脚伸展パワーは男女とも加齢とともに低下する．40代女性の体重当たりの脚伸展パワーは，70代男性とほぼ同等である（図中破線）．

従来，寝たきりや虚弱老人の発生原因として脳血管障害などの身体的要因が重視されてきたが，このような身体的要因が軽微であっても，高齢者のADLが低下する現象が最近注目されている．東京都で寝たきり高齢者を対象に行われた，自立度低下の経過に関する調査では，図1-Cに示すように，明らかな疾患・障害の発症がないままに，徐々にADLが低下する高齢者が寝たきり高齢者の約2割を占めていた[4]．また，前述したように平成13年の寝たきりの原因に関する調査でも，「高齢による衰弱」というような，疾患以外の原因が上位を占めている．

高齢者の外出が減り，日常生活における活動範囲が概ね屋内に限られてしまったような状態は「閉じこもり」と呼ばれている[5]．

閉じこもりの原因の一つはADLの障害である．厚生労働省の調査によれば，65歳以上の高齢者の20%弱，85歳以上では30%に日常生活動作や外出に支障が認められる[6]．

その一方で，総務庁の調査によれば85歳以上の高齢者では，「自分から積極的に外出する」高齢者は45%に過ぎず，逆に30%以上の方が，「誘われても外出しない」，あるいは「ほとんど外出しない」と答えており，これは高齢者が外出に消極的になりがちであることを表している（総務庁「高齢者の日常生活に関する意識調査（平成11年）」）．

現在，地域高齢者の約10%に「閉じこもり」が認められると考えられているが，実際にADLの低下など身体的な原因が主な「閉じこもり」は3,4割に過ぎず，むしろ心理的・社会的要因による「閉じこもり」が多いと推定されている[7]．

「閉じこもり」の原因は多岐にわたる（図2）．心理的要因としては，外出が怖い（尿失禁，転倒恐怖など），出かける目的がない（無趣味，退職など），家族への遠慮や依存などが挙げられる．社会的要因としては，社会的役割や家庭内での役割の喪失，対人交流の減少などが考えられる．また，家内外の環境が外出の妨げとなっていることもある．

すなわち加齢や身体的要因だけではなく，心理的・社会的要因，環境要因が，高齢者の閉じこもりを生み出し，二次的に体力や社会的適応能力を低下させると考えられる．

従って寝たきり・閉じこもりを予防するためには，身体的，精神的，社会的要因を考慮した総合的なアプローチが必要である．

健康寿命の延長にむけて

寝たきりゼロ作戦や介護保険の理念はこのような研究結果に基づいて推進されてきた．その結果，この10年間で寝たきり率は特に80歳以上の高齢者で大きく減少した．これは国家レベルで推進してきた方策が概ね誤りでなかったこと，そして，閉じこもりや疾患の予防によって，さらに自立した高齢者の割合が増えていく可能性を示している．

現在，男性の要介護期間は約1.5年，女性では2.5年と考えられている．今後は，脳血管障害や骨折などの疾患の発症よりもむしろ，加齢に伴う心身の機能低下が要介護状態の主要因となると考えられ，心身の機能低下を如何に予防するかが，高齢者のADL保持のポイントとなる．

図3は地域住民を対象とした我々の調査結果の一部で

ある．中高年者の体重当たりの脚筋力（伸展パワー）は加齢とともに低下する．特に女性の40代の脚筋力は男性の70代とほぼ同等であり，筋力を保持することは女性において，より重要だと考えられる．しかし，運動習慣の割合は20代から40代の女性で20%前後と低い（平成12年度国民栄養調査）．仕事，育児，家事などで余暇時間が少ないことが影響していると考えられるが，今後女性の要介護期間を減らす意味でも，女性の運動習慣が増えるような社会的支援が必要である．

また，藤原らの研究によれば[8]，一般地域住民においてIADLの低下に先駆けて，知的能動性や社会活動が低下する．知的関心の低下や社会参加が減ることは社会適応を低下させ，閉じこもりの原因となると考えられる．高齢者の社会参加を増やし，高齢者の生き甲斐を創世するような社会基盤の整備が，将来の要介護人口を減らし，より健康的な高齢社会を形成するために必要である．

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Abstract

Strategies to reduce bed-ridden or house-bound elderly people in Japan

Fujiko Ando

Preventive medicine is supposed to be important for reducing bed-ridden ('netakiri', in Japanese) or frail elderly people. Previous studies showed that only about 30% of the bed-ridden elderly had decreased their ADL levels directly due to diseases, such as cerebrovascular disease or hip fracture. One of the other important causes of 'Netakiri' is disused syndrome. A few weeks after staying in bed, not only muscle power but also bone mineral density and intellectual interest often decrease in the elderly. Rehabilitation in daily life is expected to prevent disused syndrome. House-bound ('tojikomori', in Japanese) is supposed to be another cause of reduction of ADL. There are miscellaneous causes of tojikomori. Aging is one of the most important factors, but cannot be modified. Physical, mental, social or environmental factors are also important. Participation in social activity, improvement of intellectual interest and habitual physical excise, as well as prevention of diseases, is expected to be useful for preventing 'tojikomori' and 'netakiri' in the elderly.

Key words: *House-bound, Bed-ridden, Frail elderly, Preventive medicine*
(Jpn J Geriat 2004；41：61—64)

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REGULAR PAPER

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Clinical and pathological studies of familial amyotrophic lateral sclerosis (FALS) with *SOD1* H46R mutation in large Japanese families

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Abstract We clarified the clinical and pathological aspects of familial amyotrophic lateral sclerosis (FALS) with *SOD1* H46R heterozygous mutation in the Miyakonojo Basin, a region in southern Japan where the prevalence of ALS is 11.4 per 10^5 of the population. We studied 17 patients, including one autopsy case, in three FALS families with the mutation. The average age at disease onset in the families was 44.3±8.7 years, and the mean disease duration was 12±7.6 years, with a range of 6 to 30 years. Ten of 17 patients were unable to walk by the mean age of 56.4±12.2 years. The initial symptom was muscle weakness in the distal leg muscle in all patients. The autopsy findings of one FALS patient showed atrophy of lateral and anterior funiculi, decreased numbers of anterior horn cells, preserved posterior funiculus and absence of neuronal inclusion bodies. Percentages of mutant *SOD1* protein measured by mass spectrometry were 14% in erythrocytes, 43% in the spinal cord, 47% in the iliopsoas muscle and 60% in the diaphragm. In this study, we confirmed that FALS with *SOD1* H46R mutation showed uniform initial symptoms and slow disease progression with intrafamilial variation of disease severity and that inclusion body formation is not essential in FALS with this mutation.

Keywords FALS · *SOD1* H46R mutation · Autopsy case · Mutant protein · Clinical course

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Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder, the cause of which remains unknown. Familial ALS (FALS) comprises about 5 to 10% of ALS [18], and about 20% of FALS showed the Cu/Zn SOD gene (*SOD1*) mutations [6, 23]. Since the discovery of *SOD1* mutations in FALS, many families with different point mutations of the gene have been reported [1, 2, 5, 13, 18, 22, 26]. Clinical features and neuropathological findings in FALS may differ from those of sporadic ALS in some respects. There have only been a few reports on the detailed neuropathological findings in FALS with Cu/Zn SOD gene mutations [5, 9, 12, 14, 21, 25, 26, 28]. It is important to investigate the relation between *SOD1* mutations and the clinical and neuropathological findings.

We conducted an epidemiological study of FALS in the Miyakonojo Basin in the southern part of Japan, which has a population of 210,297. The worldwide prevalence of ALS is 4–6 per 100,000 [8]. Some regions, such as the Kii Peninsula and Guam, have a higher prevalence of ALS [29, 30]. In 1998, the prevalence of ALS in Japan was 3.8 per 100,000, based on the number of ALS patients enrolled in the Research for Specific Diseases supported by the Ministry of Health and Welfare, Japan. Meanwhile the prevalence of ALS in the Miyakonojo Basin was 11.4 per 100,000 and was about triple the value for that of Japan as a whole [20]. Moreover, FALS patients were more predominant than sporadic ALS patients in this region. In

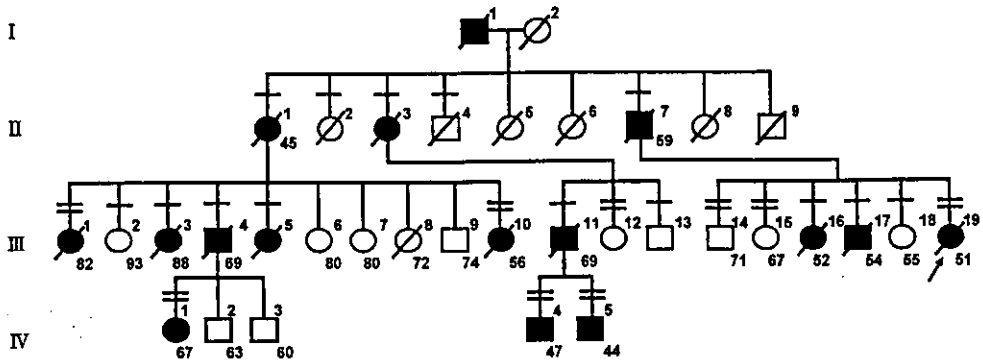

Fig. 1 One representative pedigree of three FALS families with *SOD1* H46R mutation from the Miyakonojo Basin. *Filled symbols* represent patients with ALS; *empty symbols*, healthy subjects; *oblique slash*, deceased; *lower left*, age at examination or death; *squares* represent men; *circles*, women; '-', patient observed by other hospitals; '=', confirmed by authors. The *arrow* indicates the autopsy case, a 51-year-old female at the time of death

Table 1 Clinical features of FALS patients with *SOD1* His46Arg mutation in the Miyakonojo Basin

Family no.	F-1	F-2	F-3	Total
No. of patients	4	10	3	17
Age at disease onset (years)	47.5±11.9	43.2±7.2	43.0±11.0	44.3±8.7
Disease duration (years)	9.3±7.7	14.8±7.6	8.0±6.2	12.1±7.6
Age when unable to walk (years)	61.0±0.0	59.0±13.0	46.5±9.2	56.4±12.2
Interval between onset of symptoms and respiratory failure (years)	11.5±7.8	19.2±6.6	_	17.0±7.3
Tongue atrophy (cases)	0/3	1/6	0/3	1/12
Weakness of unilateral lower limb as an initial symptom (cases)	4/4	10/10	3/3	17/17
Numbness (cases)	1/1	3/5	0/1	4/7
Dementia (cases)	0/4	0/10	0/3	0/17
Glucose intolerane (cases)	1/1	2/3	1/2	4/6

those FALS patients, a His46Arg (H46R) mutation in exon 2 of *SOD1* was detected in at least three FALS families who had no blood relationship and who were from Miyakonojo City. Interestingly, the patients with the mutation showed intra-familial clinical variations, which suggested that the disease progression was not only determined by genetic abnormality, but also by other factors such as environmental factors and other modifier gene(s). Here, we report the clinical features of three FALS families with *SOD1* H46R mutation and the neuropathological findings in one autopsy case.

Materials and methods

Patient evaluation

We studied 17 patients, including one autopsy case, from three FALS families in the Miyakonojo Basin. The patients were diagnosed as having ALS based on neurological examination, blood tests, electrophysiological study, brain CT/MRI and genetic examination using DNA obtained from peripheral blood. All patients had lower motor neuron involvement in at least two limbs with slow progression. Deep tendon reflexes were increased in only one patient. These clinical findings partially met the diagnostic criteria for ALS [24]. Mutations in the Cu/Zn SOD gene (*SOD1*) were identified by standard sequencing methods [2, 23]. All studies were performed with informed consent.

The autopsy case, a 51-year-old Japanese woman (Fig. 1, III-19), developed muscle weakness of the left lower extremity at age 34. Weakness of the right lower and upper extremities had started at age 36. She complained of dysesthesia of the left lower extremity at age 40. She became unable to walk at age 45 and had been bedridden since age 47. She had dysphagia and dyspnea at age 49. On neurological examination at the same age, she had dysarthria, dysphagia, tongue atrophy with fasciculation and muscle weakness

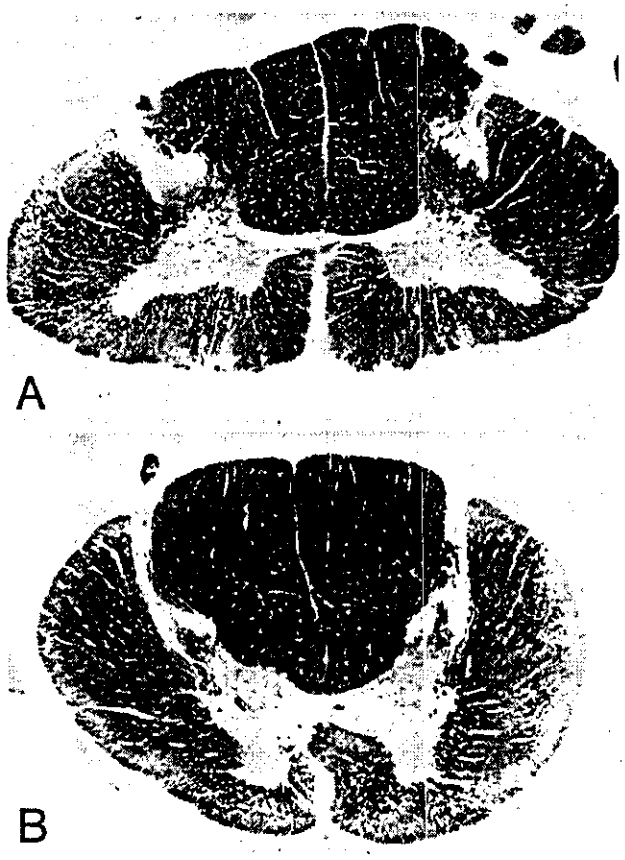

Fig. 2 The spinal cord findings at cervical (A) and thoracic (B) levels. Posterior funiculus showed no abnormality, but the anterior funiculus and spinocerebellar tracts were mildly degenerated. Klüver-Barrera, A: ×8, B: ×10

with atrophy in all four extremities. She had areflexia, but no Babinski's sign. Her consciousness, mental faculties and eye movement were normal. No sensory abnormalities were detected clinically and electrophysiologically. She began to use the artificial respirator by nasal intermittent positive pressure ventilation (NIPPV) at night. She died of lung hemorrhage at age 51 without any other bleeding or coagulation abnormality. The total disease duration was 17 years. Autopsy was performed 6 h after death.

Histopathology and immunohistochemistry

The brain and spinal cord were fixed with 10% formalin and embedded in paraffin for neuropathological study. The paraffin sections of the motor cortex, cerebellum, brain stem and spinal cord at the cervical, thoracic and lumbar levels were cut at 5-μm thickness and stained by the following routine methods: hematoxylin and eosin (H-E) and Klüver-Barrera (K-B). Immunocytochemical examination was performed using the following antibodies: an affinity-purified rabbit polyclonal antibody against human ubiquitin (DAKO, 1:1,000), a mouse monoclonal antibody against human glial fibrillary acidic protein (GFAP; Chemicon International, Inc., 1:100) and an affinity-purified goat polyclonal antibody against human *SOD1* (C-17 and N-1, Santa Cruz Biotechnology, Inc., 1:100). Sections were deparaffinized, then endogenous peroxidase activity was quenched for 30 min with 0.3% H_2O_2 and washed in phosphate-buffered saline (PBS, pH 7.2). Normal sera homologous with the second antibody were used as blocking reagents. Sections were incubated with the antibodies overnight at 4°C. A standard avidin-biotin complex method was used for the secondary antibody step (Vectastain, Elite, Vector Labs).

Immunoprecipitation of *SOD1*s and on line liquid chromatography-electrospray ionization mass spectrometry (LC-ESIMS) analysis

Immunoprecipitation was performed as described previously [13] with slight modification. In brief, tissues obtained upon autopsy were homogenized in an ice bath with twice the weight volume of saline and were centrifuged (23,500 g) to remove the insoluble fraction. Erythrocytes were lysed with a mixture of four volumes of double-distilled water, and cell debris was removed by centrifugation (23,500 g). Forty microliters of antibody against human *SOD1* (The Binding Site, Birmingham, UK) was mixed with 40 μl of the soluble fraction of the homogenate and the haemolysate. After 2 h, the antigen-antibody complexes were collected by centrifugation (23,500 g), washed in cold saline three times and dissolved in 25 μl of 2% acetate containing 100 mM dithiothreitol to separate the antibody and *SOD1*. After 15 min, 10 μl of the sample solution was injected into a LC-ESIMS system. LC-ESIMS analysis was performed as described previously [17].

Results

Clinical features of patients with *SOD1* H46R mutation

In 17 patients of three FALS families, the ages at disease onset ranged from 32 to 60 years (average 44.3±8.7 years), the ages at death from 46 to older than 88 years (average 58.8±13.7 years) and the mean disease duration was 12.1±

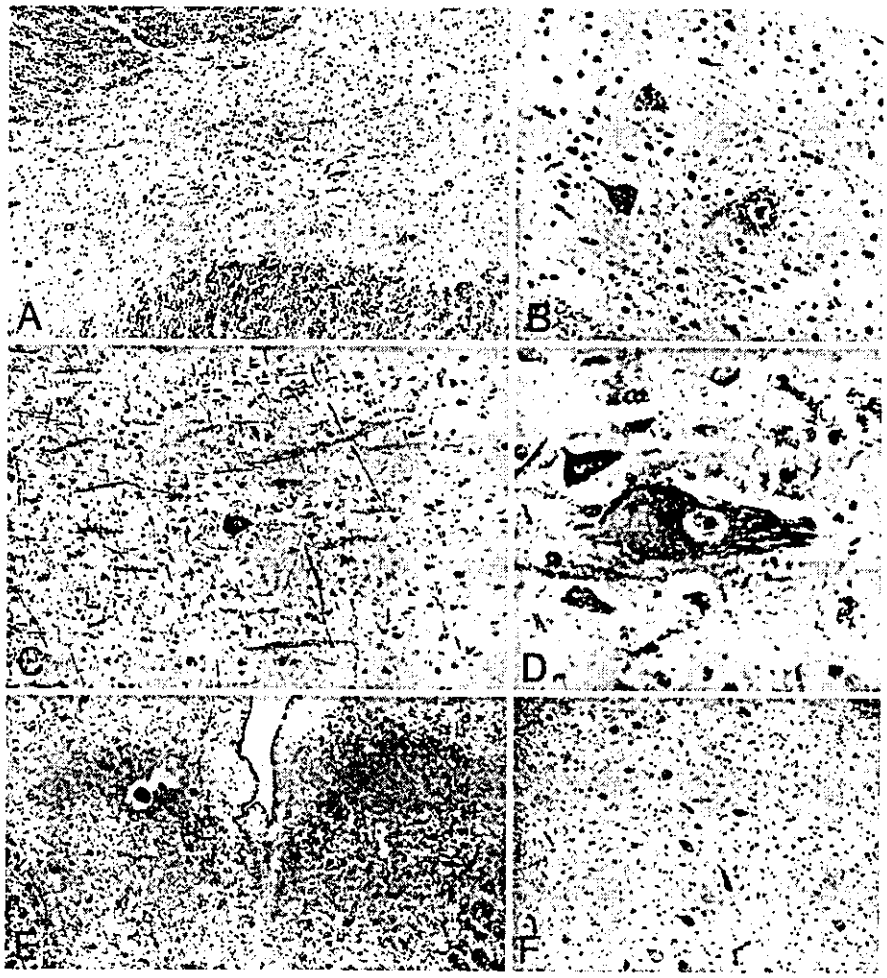

Fig. 3 Anterior horn findings of spinal cord at the cervical level. **A** Numbers of motor neurons were obviously decreased in the anterior horn. Klüver-Barrera, ×50. **B** The remaining motor neurons were observed to be normal. Neither Bunina nor Lewy body-like inclusion bodies were present in the neurons. We could not find any inclusion bodies in the sections immunostained with antibodies against ubiquitin and *SOD1*. HE, ×225. **C** The structure of the motor cortex was well preserved. Klüver-Barrera, ×25. **D** Mild and partial chromatolytic changes were seen only in a few Betz cells. Klüver-Barrera, ×250. **E** Gliosis was seen in the hypoglossal nucleus. Klüver-Barrera, ×25. **F** The reactive astrocytes increased in the hypoglossal nucleus. HE, ×55

7.6 years. The initial symptom was muscle weakness of a unilateral lower limb in all patients. The mean interval between onset of symptoms and some sign in the upper extremities was 6.6±4.5 years. The mean age when they were unable to walk was 56.4±12.2 (n=10). The mean interval between onset of symptoms and inability to walk was 9.9±6.4 years. The mean interval between the onset of symptoms and respiratory failure was 17.0±7.3 years (n=7). Tongue atrophy was found in only one patient. Deep tendon reflexes increased in one case and decreased in four cases at the time of our examination. None of the patients showed Babinski's sign or dementia. Four patients had numbness in their extremities, but sensory disturbance was not detected by neurological examination. Four of six cases examined by OGTT showed glucose intolerance (Table 1).

Pathological findings

Macroscopic findings

The 51-year-old FALS patient showed diffuse muscular atrophy in all four extremities. The right lung showed acute congestion, but there were no significant findings in the left lung. There were no other bleeding sites or vascular abnormalities. There were no abnormalities in the other visceral organs. Coronal sections of the brain were unremarkable. The spine showed only atrophy of the anterior roots of the spinal cord.

Microscopic findings

Lateral and anterior funiculi of the spinal cord showed atrophy, but the posterior funiculus was preserved. Spinocerebellar tracts were particularly degenerated in the lateral funiculus (Fig. 2A, B). In the anterior horn, the number of anterior horn cells decreased, and there was absence of neuronal inclusion bodies in the remaining cells (Fig. 3A). Neither Bunina nor Lewy body-like inclusion bodies were present in the neurons (Fig. 3B). Immunostaining with antibodies against ubiquitin or *SOD1* also showed no inclusion bodies in the cells. The structure of the motor cortex was well preserved (Fig. 3C). Mild and partial chromatolytic changes were seen only in a few Betz cells (Fig. 3D). Gliosis was seen in the hypoglossal nucleus (Fig. 3E, F). The oculomotor nucleus, Clarke's column, intermediolateral column, Onufrowicz nucleus and posterior root ganglion appeared normal (Fig. 4A–D). The cerebellar cortex appeared normal.

LC-ESIMS analysis

Percentages of mutant *SOD1* protein measured by the LC-ESIMS system using various tissues from the autop-

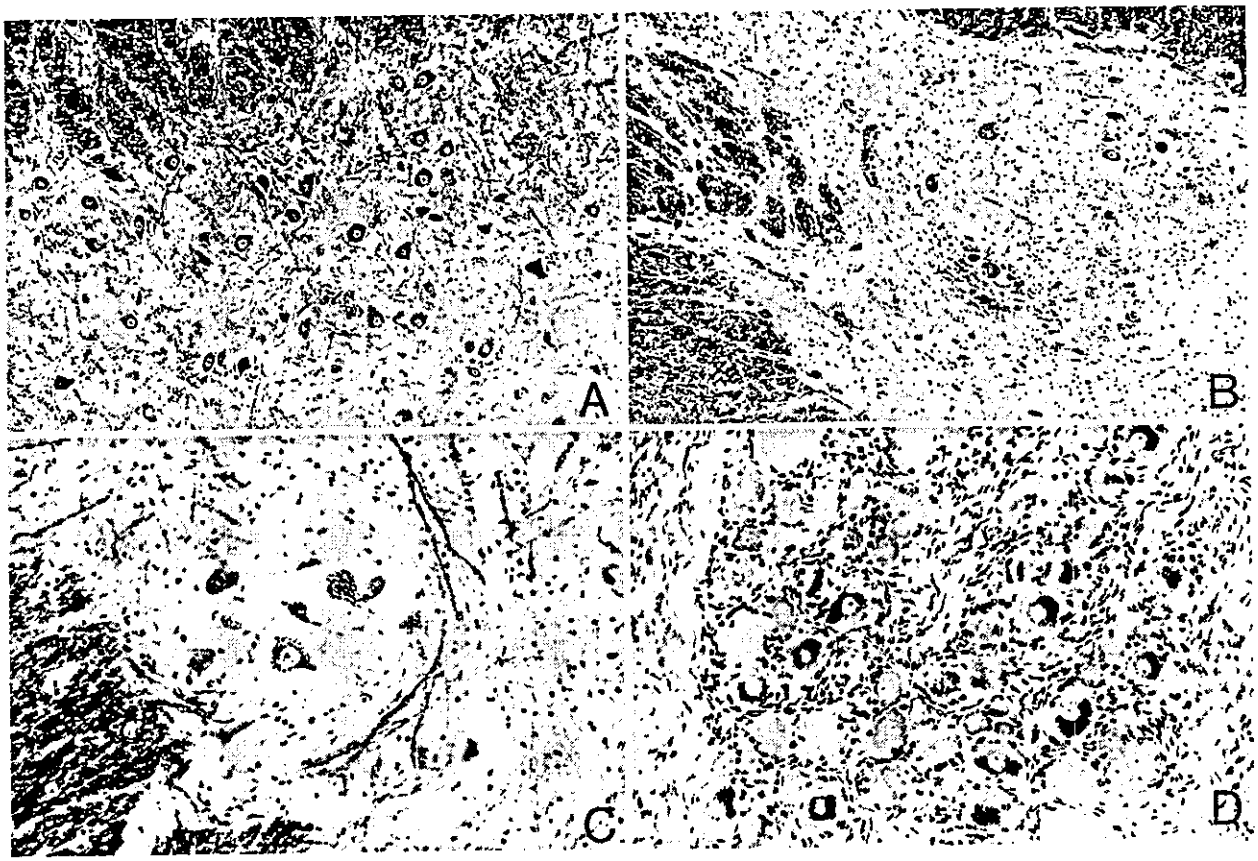

Fig. 4 Oculomotor nucleus (A: ×63), Clarke'nucleus and intermediolateral column (B: ×63), Onufrowicz nucleus (C: ×127) and posterior root ganglion (D: ×135) showed no degeneration. Klüver-Barrera

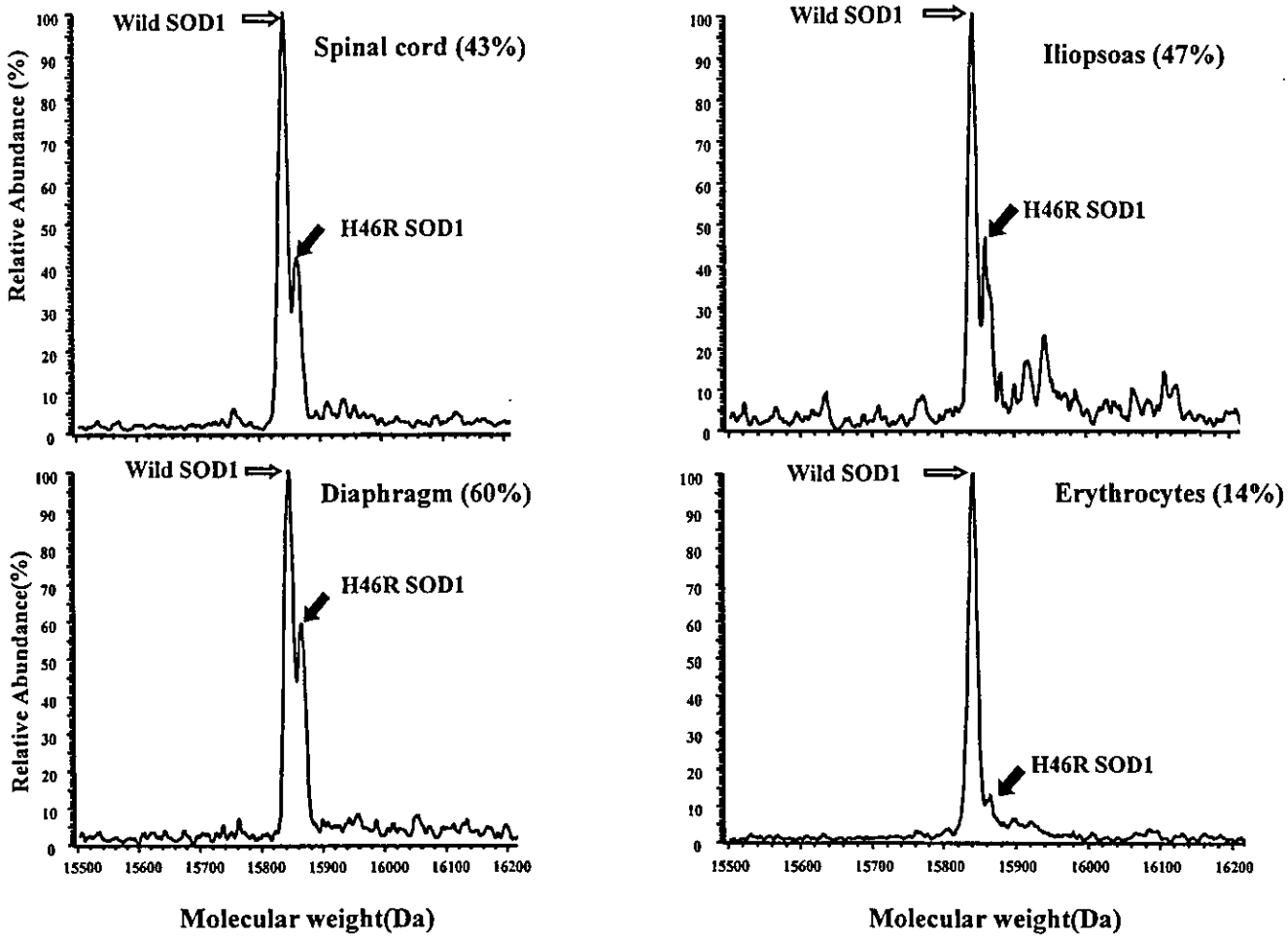

Fig. 5 Mass spectra of human *SOD1*s immunoprecipitated from various tissues from this patient with *SOD1* H46R mutation. Percentages of mutant *SOD1* protein were 43% in the spinal cord, 47% in the iliopsoas muscle, 60% in the diaphragm and 14% in erythrocytes

sied patient were 43% in the spinal cord, 47% in the iliopsoas muscle, 60% in the diaphragm and 14% in erythrocytes (Fig. 5).

Discussion

We have diagnosed these familial cases as having ALS based on clinical and neuropathological findings. Our previous study showed that the prevalence of ALS in the Miyakonojo Basin was three times higher than that seen in Japan as a whole[20]. Therefore, we speculated that ALS patients were clustered in this region, though the prevalence was not as high as that in the Kii Peninsula[29]. This region has been developed as one cultural area since ancient times, and the cultural exchange with places outside of the basin might have been limited. From this historical background, FALS may have accumulated in this area. Our genetic study showed that most of the FALS in the Miyakonojo Basin had the *SOD1* H46R mutation. In this study, we analyzed the clinical features and neuropathological findings in FALS with *SOD1* H46R mutation in this area.

The characteristic clinical features of FALS with H46R mutation were muscle weakness in the distal part of the lower extremity as the initial symptom, slow progression, relatively rare cranial nerve involvement and transient upper motor neuron signs. The disease progression in FALS with H46R mutation was rather slow in comparison with other types of *SOD1* mutations [1, 3, 19], but the disease severity was different if inter- or intra-family. The symptoms in the upper extremities appeared between 2 and 15 years, and respiratory failure appeared 6 years at the earliest and 30 years at the latest after the initial symptoms in the legs appeared. The intervals between the initial symptoms and inability to walk were also varied, as shown in Table 1. More interestingly, some pairs of parents and children showed remarkable differences in ages at disease onset. These data suggest that some environmental and other genetic factor(s) are related to the onset and progression of the disease in addition to the genetic background.

In this paper we reported the detailed neuropathological findings of a patient with H46R *SOD1* mutation and compared the pathological findings with those in other *SOD1* mutations: A4T [28], A4V [5], H48G [26], E100G[9], I113T [21, 25], TTG126xxG [12] and H46R [19] (Table 2). The characteristic findings in our patient

Table 2 Comparison of pathological findings of patients with the *SOD1* mutation. – No degeneration, ± slight degeneration, + degeneration, *NS* not stated, * the mean age of onset, ** the mean disease duration

Authors	*SOD1* mutation	Age	Duration (months)	Cortico-spinal tract	Anterior horn cell loss	Inclusion body	Posterior funiculus	Clarkes nucleus	Spino cerebellar tract	Onufrowicz nucleus	Betz cell	Posterior root ganglion
Takahashi et al.	A4T	40	9	±	+	+	+	+	+	–	–	–
Cudkowicz et al. [5] (5 cases)	A4V	45.4*	12**	+	+	+	+	+	+	NS	–	NS
Motizuki et al. (unpublished)	H43R	58	7	+	+	+	+	+	±	NS	±	NS
Shaw et al. [26]	H48G	54	9	+	+	+	±	NS	+	NS	+	NS
Ince et al. [9]	E100G	36	39	+	+	+	+	+	+	–	–	NS
Orrell et al. [21]	I113T	48	252	+	+	–	–	+	+	–	NS	NS
Rouleau et al. [25]	I113T	63	24	–	+	+	NS	NS	NS	NS	–	NS
Kato et al. [12]	TTG126 xxG	46	18	+	+	+	+	–	–	–	NS	–
		65	132	+	+	+	+	+	+	+	NS	+
Ohi et al. [19]	H46R	70	480	+	+	–	±	+	+	–	NS	NS
This case	H46R	51	204	±	+	–	–	–	+	–	±	–

with *SOD1* H46R with 17-year disease duration are absence of inclusion bodies in the cell, very mild change in the lateral funiculus, loss of anterior horn cells at any level of the cervical, thoracic, lumbar, and sacral spinal cord and no degeneration in the posterior funiculus, Clarke's nucleus, posterior root ganglion or Onufrowicz nucleus. Although Ohi et al. have reported that the posterior funiculus was slightly degenerated and the number of neurons in Clarke's column was reduced in their patient with *SOD1* H46R mutation and 40-year disease duration [19], this difference might be related to the longer disease duration in their patient. Interestingly, our autopsied patient showed spinocerebellar tract degeneration and severe loss of anterior horn cells, but very mild changes in the corticospinal tract. This indicates that the lower motor neuron was more degenerated than the upper motor neuron in this patient. These spinal cord findings were similar to those of spinal progressive muscular atrophy (SPMA) and constitute the clinical features in the FALS families with *SOD1* H46R mutation in the Miyakonojo Basin. In addition to the above spinal cord findings, we were the first to describe the central nervous system findings in FALS with *SOD1* H46R mutation that were not described by Ohi et al. [19]. We observed almost normal Betz cells in the motor cortex, but gliosis in the hypoglossal nucleus that was related to her tongue atrophy. No gliosis was found in the oculomotor nucleus, although Kato et al. have reported mild neuronal loss with gliosis in the nucleus in the patient with TTG126xxG mutation [12].

Neural cell inclusion bodies are usually found in FALS with *SOD1* mutation [9, 11, 12, 27, 28], but no inclusion bodies were found in our and in previously reported patients, regardless of intensive histological survey [19]. These data suggest that inclusion body formation is not always concerned in the etiology of FALS with *SOD1* H46R mutations.

Histidine 46 is located in the active site of *SOD1*, near the binding site for copper [14]. The H46R mutation in this site must affect *SOD1* dysfunction in a different way compared to other mutation sites and may induce the characteristic clinical features that we have shown in this study. A transgenic rat expressing the mutated human *SOD1* (H46R) gene showed more mutant *SOD1* product, less enzymatic activity of the *SOD1* and slower disease progression than occurred in other mutations [16]. These findings in the animal model were similar to those seen in patients with *SOD1* H46R, although the animal model showed inclusion bodies.

We studied the mutant *SOD1* protein concentration in several tissues obtained from the autopsied patient with *SOD1* H46R mutation. The relative amounts of mutant *SOD1* to normal *SOD1* are highest in the diaphragm, followed by the spinal cord and skeletal muscle, and lowest in erythrocytes. If the same analysis was done using isolated anterior horn cells, mutant *SOD1* concentration would probably be higher than that in the entire spinal cord. This mutant *SOD1* protein may be involved in the motor neuronopathy in FALS. Mourelatos et al. have reported that the fragmentation of the Golgi apparatus (GA) of motor

neurons in ALS represented an early change of the organelle that may be involved in the pathogenesis of ALS [15]. The fragmentation of GA, however, was seen not only in ALS, but also in corticobasal degeneration and Creutzfeldt-Jakob disease [7]. Therefore, GA fragmentation is not specific to motor neurons in ALS. Recently, on the 'gain of function' hypothesis, several reports suggest that mutant *SOD1* proteins or intermediate filament aggregates cause an impediment in axonal transport, mitochondrial dysfunction or ubiquitin proteosome dysfunction and lead to motor neuron death in ALS [4, 10]. It is critical to clarify how the mutant *SOD1* proteins are involved in motor neuron death in FALS with no inclusion body.

In conclusion, we confirmed that FALS with *SOD1* H46R mutation showed uniform initial symptoms and slow disease progression with intra-familial variation of disease severity and that inclusion body formation is not necessary for disease onset and progression of ALS with this mutation.

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SHORT REPORT

Early pathological changes in the parkinsonian brain demonstrated by diffusion tensor MRI

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See Editorial Commentary, p 352

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Objective: To determine whether the fractional anisotropy (FA) of magnetic resonance diffusion tensor imaging is decreased in the nigrostriatal projection in parkinsonian patients.

Methods: FA values were compared in the extrapyramidal system of 12 patients with Parkinson's disease and eight age matched normal controls.

Results: Patients with Parkinson's disease had significantly decreased FA in the region of interest along a line between the substantia nigra and the lower part of the putamen/caudate complex, in which most of the nigrostriatal dopaminergic neurones are included. Loss of FA in this region was obvious even during the early clinical stages of Parkinson's disease.

Conclusions: Assuming that the loss of FA parallels the neuronal change in the brain, the results are consistent with the view that more than half the dopaminergic neurones in the nigrostriatal projection are lost before the onset of Parkinson's disease. Close comparison of FA in the basal ganglia may contribute to the early diagnosis of Parkinson's disease.

The discovery of the selective loss of dopaminergic neurones projecting from the substantia nigra in the midbrain to the neostriatum in patients with idiopathic Parkinson's disease has led to the use of l-dopa treatment, which markedly improves the prognosis of parkinsonian patients.[1 2]

According to pathological studies, at least 70–80% of dopaminergic neurones were lost before the onset of Parkinson's disease.[3] A significant amount of dopaminergic neurone loss during the early stages of Parkinson's disease was also suggested by functional imaging of these neurones using positron emission tomography (PET) with L-[^{18}F]-fluorodopa,[4] or single photon emission computer tomography (SPECT) with [^{123}I]-βCIT.[5]

Recently, magnetic resonance (MR) diffusion tensor imaging of the brain has allowed visualisation of neuronal projections in the central nervous system,[6] or estimation of the neuronal changes in the white matter of either normal subjects[7] or patients with neurological diseases such as amyotrophic lateral sclerosis[8] and multiple sclerosis,[9] through a decrease in the fractional anisotropy (FA) value derived from the MR tensor image. In the present study, we compared FA values in the extrapyramidal system of normal subjects with those of parkinsonian patients.

METHODS

Twelve patients with Parkinson's disease (mean (SD) age, 71.3 (7.7) years) were compared with eight age matched normal subjects (70.1 (8.4) years). Seven patients with progressive supranuclear palsy (PSP) (74.6 (7.3) years) were also studied. As patients with PSP show brain atrophy, including atrophy of the basal ganglia, it is suggested that they may serve as positive controls for FA change showing a robust decrease.

Parkinson's disease was diagnosed using the UK Brain Bank criteria.[10] The patients were separated into two groups, depending on the Hoen and Yahr rating scale[11]: patients in stage I or II were classified as the earlier group (n = 7; 70.0 (3.7) years), and those in stage III or more were classified as the advanced group (n = 5; 73.2 (2.4) years).

The diagnosis of PSP was according to the NINDS-SPSP criteria.[12] Normal subjects were examined by neurologists, and no abnormality was confirmed.

Informed consent for the study was obtained from each subject before the examination. MR imaging (MRI) was done with a 1.5 T imager ("Gyroscan Intera", Philips Medical Systems, Best, Netherlands), between August 1 2001 and October 31 2002. In addition to conventional T1 weighted images (repetition time (TR) 611 ms, echo time (TE) 13 ms) and T2 weighted images (TR 4754, TE 100), diffusion tensor images (DTI) were obtained. The imaging sequence of DTI was as follows: field of view 230×230 mm; matrix 128×37 with SENSE; SENSE reduction factor = 2 (matrix is 128×74 equivalent); TR/TE = 6000/88; flip angle = 90°; two b values (0 and 800 s/mm^2); 36 slices; 3/0 mm slice/gap. Diffusion sensitisation was done in six directions.[13]

FA values of 15 regions of interest (ROI) from a single hemisphere were compared between normal subjects and the patient groups. The investigators (KY and YN) were blinded to the patient's name and group. ROIs were determined (see below) on conventional MRI, and the mean FA values in the corresponding DTI were measured.

According to the landmarks, four ROIs were allocated on MRI plane "b" and "c" (fig 1A, 1C, and 1D): oval ROIs sized 41 mm^2 in the caudate head, putamen, nucleus ventralis lateralis, and subcortical white matter of the premotor cortex (area 6).

The remaining 11 ROIs were set on an axial MR image in the basal ganglia (plane "a" in fig 1A and 1B). The plane immediately below the AC–PC line roughly included structures such as the substantia nigra, subthalamic nucleus, and the lower part of the globus pallidus, putamen, and caudate nucleus.[14] This plane is also suggested to include most of the nigrostriatal neurones.[15 16] Eleven oval ROIs sized 20 mm^2 were arrayed along a line between the substantia nigra and the caudate/putamen complex at the same interval so that the ROIs covered the majority of the dopaminergic neurones between the structures (fig 1B).

To avoid the influence of motion, the subject's head was firmly fixed to the holder during acquisition. In addition, we

Abbreviations: DTI, diffusion tensor image; FA, fractional anisotropy