Ⅲ 資料

＜シンポジウム　(4)-17-1 ＞ ALS におけるコミュニケーション障害とその対策：
完全閉じ込め状態への挑戦

ALS におけるコミュニケーション障害の臨床像

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要旨：筋萎縮性側索硬化症（ALS）で眼球運動をふくめすべての随意運動が不可能になると完全閉じ込め状態（totally locked-in state; TLS）となる．TLS での意思疎通の手段を考慮するため，TLS の臨床像，頭部 MRI，SPECT，誘発脳波について検討した．TLS は呼吸器装着までの期間が，約 1.5 年で他の ALS より短かった．頭部 MRI では広範な脳萎縮をみとめ運動系を超えた広範な病変を示した．しかしながらもっとも高度な脳萎縮例でも後頭葉は残り，SPECT での後頭葉の取り込みの保存，VEP で P100　の出現などみとめることから，機能的にも視覚路が保たれることを示唆した．TLS で意思伝達を可能にする手段として視覚路を利用する方法が有望である．
（臨床神経 2013;53:1393-1395）

Key words：筋萎縮性側策硬化症，完全閉じ込め状態，人工呼吸器，意思伝達，ブレインマシンインターフェイス

はじめに

筋萎縮性側策硬化症（ALS）は一次および二次運動神経が変性消失する神経疾患である．運動神経変性の進行は運動機能を損なうばかりでなく，運動機能に依存した意思伝達能力を大きく損なうこととなる．運動障害がいちじるしく進行し，眼球運動をふくめすべての随意運動が不可能になると意思伝達不能な完全閉じ込め状態（totally locked-in state; TLS）となる[1)]．TLS にたいして意思伝達手段を確保するための試みは今まで成功しているとはいい難い．今回は臨床像から TLS の特徴を明らかにし，TLS での意思伝達の可能性について考えた．

方　法

意思伝達手段と ALS の運動障害の程度との関連を理解するために，われわれは ALS の意思伝達能力を I から V の stage に分けて検討してきた[2)]．I が文章レベルで意思伝達可能で V はいかなる手段でも意思伝達不可能という意思伝達障害の軽い方から重い方にむけての stage 分類である．今回，37 例の ALS 症例について意思伝達の障害度分類を試みた．その中で stage V に該当する症例は 10 例あった．Stage V の患者はすべて TLS 患者であった．その TLS　患者 10 例について臨床経過と意思伝達能力の stage との関連を検討した．また頭部 MRI，SPECT，誘発電位について検討した．

結　果

意思伝達能力の stage と臨床経過を検討した結果，stage V に該当する ALS 患者は呼吸器装着にいたるまでの期間が，平均で 1.5 年と短く stage　I から IV までが 3 ～ 4 年の経過があることと比較して短かった．一方，罹病期間は stage 間での差がなかった．頭部 MRI では，後頭葉を除くほとんどすべての大脳，脳幹の萎縮を示した（Fig. 1）．ただその萎縮の程度には症例間で差をみとめ，ほとんど萎縮をみとめない症例もある一方（Fig. 1a），後頭葉以外きわめて高度な萎縮をしめす症例もあった（Fig. 1f）．SPECT では，MRI での脳萎縮の強い例で後頭葉以外の著明な取り込み低下をみとめた．誘発脳波では，SEP で半数の症例で N20 が消失する一方，VEP で検討されたすべての症例で P100 をみとめた（Fig. 2）．

考　察

今回の検討で意思伝達能力の stage　V に該当する患者はすべて眼球運動をふくめ，随意運動が障害され TLS であった．TLS では呼吸器装着までの期間が短いことがわかったが，これははじめて TLS を報告した林らの結果と一致するものだった[1)]．一方罹病期間では各 stage 間で差がなかったことは，TLS は ALS が長期経過した結果ではなく，ALS の中で TLS にいたる群，いたらない群が存在することを示唆した．頭部 MRI では，TLS で広範な脳萎縮をみとめたことは，運動系を超えた病変の広がりを示唆した．逆にもっとも高度な脳萎縮例でも後頭葉は残り，その症例で SPECT での後頭葉の取り込みの保存，VEP で P100 の出現などみとめることから，機能的にも視覚路が保たれることがしめされた．感覚路の利用だけでは実際意思伝達は難しく高次脳機能はどうであるかが問題である．TLS の高次脳機能に関しては，高度の脳萎縮をみとめることから高度の高次脳機能障害が想定され

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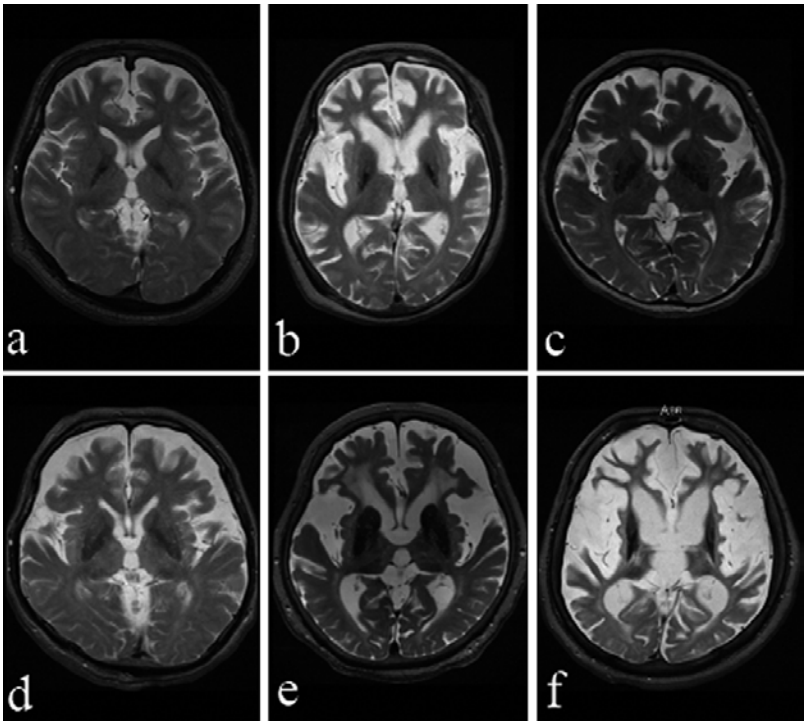
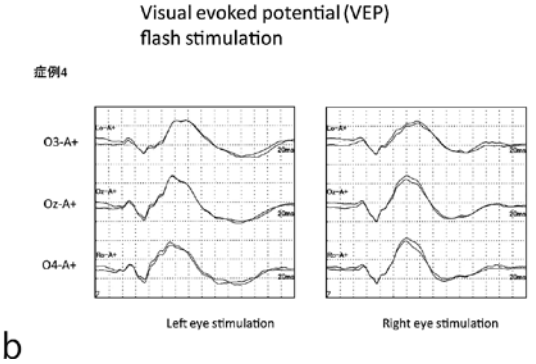


Fig. 1　TLS の頭部 MRI.
左上から右下にかけて 6 症例を脳萎縮の軽度から重度の順で表示している．a の症例はやや前頭葉萎縮を示すもののほぼ正常といえるが，その他の症例は後頭葉を除き萎縮をみとめた．f の症例では後頭葉以外はきわめて高度な萎縮をみとめた．

誘発脳波

	症例1	症例2	症例3	症例4	症例5	症例6
SEP N20	誘発なし	誘発なし	誘発なし	誘発なし	誘発あり	誘発あり
ABR V	誘発あり			誘発なし	誘発あり	誘発あり
VEP				誘発あり	誘発あり	誘発あり
EEG α 波の出現	なし		なし	なし		なし

a



b

Fig. 2　TLS の誘発脳波.
a；6 症例の誘発脳波の結果．b；症例 4 では SEP，ABR では誘発はみとめられなかったが VEP は誘発された．なお，この症例 4 の頭部 MRI は Fig. 1 の f である．

るが評価ができないのが現状である．光トポグラフィーをもちいて脳活動を検討した比較的脳萎縮の軽度な TLS の症例では高次脳活動がおこなわれていることを示唆した[3]．TLS で手段の確保により意思伝達ができるの可能性があると考えられる．

結　論

TLS では運動系ばかりではなく，体性感覚路，聴覚路も障害される一方，視覚路が保たれていた．そのことから TLS での意思伝達を可能にする手段として視覚系を利用する方法が有望であると考えられた．

※本論文に関連し，開示すべき COI 状態にある企業，組織，団体はいずれも有りません．

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Abstract

Clinical feature of ALS with communication disturbance; the possibility to communication in TLS

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In the subsets of amyotrohic lateral sclerosis (ALS), totally-locked in state (TLS) is shown as the result of marked progression of motor neuron degeneration. In TLS, patients are impossible to move any voluntary muscles. As the result, patients with TLS cannot communicate with any augmentative and alternative communication devices(AACD) at present. To find the AACD that enables for TLS to communicate, we examined the clinical character, brain MRI, SPECT and evoked potentials in TLS. Brain MRI showed marked brain atrophy including the brainstem, but the occipital lobe was spared. SPECT and visual evoked potentials (VEP) showed preserved physiological function of the occipital lobe in TLS. The results suggest that neuronal degeneration in TLS is not restricted to motor system, but that the visual pathways are spared. Patients with TLS may be possible to use AACD that utilize the visual pathway.

(Clin Neurol 2013;53:1393-1395)

Key words: amyotrophic lateral sclerosis, totally locked-in state, respirator, communication, brain machine interface

＜シンポジウム　(4)-17-2 ＞ALS におけるコミュニケーション障害とその対策：
完全閉じ込め状態への挑戦

ALS におけるコミュニケーション障害の予測因子

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要旨：気管切開・人工呼吸器（TPPV）下にある筋萎縮性側索硬化症患者 76 例において，意思伝達障害の進行に関する予測因子を検討した．意思伝達可能（stage Ⅰ），困難（stage Ⅱ～Ⅳ），不能（stage Ⅴ）の 3 群間において TPPV 装着まで期間，経管栄養まで期間，完全四肢麻痺までの期間で有意差がみとめられた．予後予測因子として，stage Ⅱへの進展因子は，発症後 2 年以前の呼吸器装着と完全四肢麻痺，stage Ⅴへの進展因子は発症後 2 年以前の眼球運動障害出現が検出された．したがって，呼吸器装着，完全四肢麻痺までの進行速度が速いこと，また眼球運動出現までの速度が速いことが，意思伝達障害を予測する因子となりうる．

（臨床神経 2013;53:1396-1398）
Key words：筋萎縮性側索硬化症，意思伝達障害，意思伝達能力の程度に基づくステージ分類，意思伝達障害予測因子

はじめに

気管切開・人工呼吸器（tracheostomy positive pressure ventilation; TPPV）を導入した筋萎縮性側索硬化症（ALS）患者の多くは意思伝達能力障害をきたす．しかし，予めその進行経過を示す指標は少なく，進行に影響する因子も不明である．著者らは意思伝達能力による分類（Stage Ⅰ：文章にて意思表出が可能，Stage Ⅱ：単語のみ表出可能，Stage Ⅲ：yes/no のみ表出可能，Stage Ⅳ：残存する随意運動はあるが yes/no の確認が困難なことがある，Stage Ⅴ：全随意運動が消失して意思伝達不能な状態＝完全閉じ込め状態：Totally locked-in state: TLS）を作成して，TPPV を導入された ALS 剖検例の臨床経過を検討し，stage Ⅴにいたった症例は発症 2 年以内と早期に TPPV が導入された事を報告した[1)]．そこで，さらに，生存例を加えて TPPV 下の ALS 患者の経過を検討し，TPPV 導入後に意思伝達に影響を与える因子を明らかにすることを目的とした．

対象および方法

TPPV 下の ALS 剖検 29 例および，2005 年から 2012 年まで経過観察しえた TPPV 下 ALS 患者 47 例の計 76 例を対象とした．診療録・看護記録を後方視的に調査し，生存例については担当の看護師への聞き取り調査を加えた．意思伝達能力の程度に基づく stage 分類[1)]に沿って各症例を分類した．

3 群比較：意思伝達可能（stage Ⅰ），困難（stage Ⅱ～Ⅳ），不能（Ⅴ）の 3 群間で以下の項目について χ 二乗検定，Kruskal-Wallis 検定をもちいて比較した．性別，家族歴・遺伝子変異の有無，発症年齢，発症部位，罹病期間，経管栄養の有無，眼球運動障害の有無，四肢完全麻痺の有無および，発症からの 1．TPPV 装着までの期間，2．経管栄養と導入までの期間，3．眼球運動障害出現までの期間，4．完全四肢麻痺までの期間．

予測因子の検討：発症後 2 年時点での TPPV 装着，経管栄養導入，眼球運動障害出現，完全四肢麻痺の有無によって 2 群にわけ，stage 進展をイベントとし，Kaplan-Meier 解析・log-rank 検定をおこなった．さらに，調査項目を従属変数として，Cox 比例ハザードモデル（単変量で有意な項目を従属変数にした多変量解析）で解析した．なお，生存例については，2012 年 4 月 1 日を打ち切り日とした．

統計学的解析には，PASW Statistic ver.19.0（IBM SPSS Statics）をもちい，$p < 0.05$ を統計学的有意差とした．なお本研究は，筆頭者所属機関の倫理委員会の承認をえておこない，対象の匿名化，ID による管理などデータ管理に細心の注意を払い，個人が特定されることのないよう務めた．

結　果

全 76 例の発症年齢は，13 歳から 73 歳（中央値 54.0，四分

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Table 1　Clinical characteristics of each endpoint stage.

Stage	Stage I	Stage II–IV	Stage V	p-value
Number of patients	36 (47.3%)	27 (35.5%)	13 (17.2%)	
Sex (male)**	23 (63.4%)	19 (70.3%)	6 (46.1%)	N.S.
Familial or gene mutation**	3 (8.3%)	2 (7.4%)	4 (30.7%)	N.S.
Age of onset (years)*	54.5 (47.3–61.0)	56.0 (49.0–68.0)	52.0 (38.5–60.5)	N.S.
Total disease duration (months)*	104.0 (59.3–168.8)	86.0 (57.0–199.0)	126.0 (83.5–171.0)	N.S.
Duration from the onset				
to TPPV (months)*	56.6 (29.8–82.0)	21.0 (17.0–36.0)	15.0 (12.5–25.5)	<0.0001
to tubefeeding (months)*	56.0 (29.8–93.5)	25.0 (15.0–61.0)	16.0 (12.0–29.0)	0.001
to opthalmoplegia (months)*	84.0 (46.0–147.0)	50.0 (29.5–115.0)	33.0 (16.0–69.0)	N.S.
to quadriplegia (months)*	78.0 (55.0–183.0)	65.0 (43.5–126.0)	31.0 (22.0–55.5)	0.002

median (IQR: interquartile range)
TPPV: tracheostomy positive pressure ventilation
N.S.: No Significant
*: Kruskal-Wallis　**: Chii-square test

位範囲 44.3～63.8），罹病期間 28～371 ヵ月（104.0，61.5～169.3）であった．3 群間で性，家族歴・遺伝子変異の有無，発症年齢，罹病期間に有意差はなかったが，発症から TPPV 装着までの期間，発症から経管栄養導入までの期間，発症から完全四肢麻痺までの期間，眼球運動障害の出現と完全四肢麻痺者の割合に有意な差がみとめられた（Table 1）．

さらに，Kaplan-Meier 解析において，発症 2 年時点での TPPV 装着，経管栄養導入，眼球運動障害出現，完全四肢麻痺が生じた群は，生じていない群より，有意に早く Stage II および Stage V にいたった（$p < 0.01$）．

次に，Cox の比例ハザードモデルによる解析では，stage II への進展については呼吸器装着（ハザード比 8.018，$p < 0.05$）と完全四肢麻痺（ハザード比 23.798，$p < 0.01$）に有意差がみとめられた．また，stage V への進展では，眼球運動障害出現まで期間（ハザード比 7.034，$p < 0.05$）に有意差がみとめられた．

考　察

今回の分析によって，意思伝達困難（Stage II～IV）へいたるには発症 2 年時点での TPPV 装着と完全四肢麻痺が，一方，意思伝達不能（Stage V）へは，眼球運動障害出現がリスク因子としてあげられた．TPPV 装着後の ALS 患者の意思伝達手段には，眼球運動や上下肢のわずかな動きがもちいられるばあいが多い[2)3)]．完全四肢麻痺になると，スイッチ操作に必要なわずかな動きすら途絶えるために意思伝達困難になり，眼球運動障害のために yes-no の合図が途絶えることで意思伝達不能にいたる可能性が高いといえる．

人工呼吸器装着後の ALS 患者にとっていわゆる「生活の質（QOL）」を左右するもっとも大きな問題は，コミュニケーション障害といえる[4)]．一般に ALS では，発症 2～4 年が，生存中央値とされており[5)]，発症 2 年時点でのリスク因子を明らかにすることは，その後の治療・療養方法に関する自己決定やケア介入，さらにブレインマシーンインターフェーズを適切な時期に導入する[6)]ために有用であると考えられた．

結　論

ALS 患者では，TPPV 装着まで・完全四肢麻痺までの進行速度が速いこと，また眼球運動障害出現までの速度が速いことが，意思伝達障害を予測する因子となりうることが明らかになった．症状進行を予測して，ブレーンマシーンインターフェーズ導入を含んだ適切なケア介入を検討する必要がある．

※本論文に関連し，開示すべき COI 状態にある企業，組織，団体はいずれも有りません．

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Abstract

Predictors the progression of communication impairment in ALS Tracheostomy Ventilator users

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We investigated predictive factors associatied with progression of communication impairment in 76 patients with amyotrophic lateral sclerosis (ALS) using tracheostomy ventilation. We classified the patients into the following three groups: patients capable of communication (stage I), patients with difficulties in communication (stage II to IV), and patients incapable of communication (stage V: so-called totally locked-in state) (Hayashi, et al. Clin Neurol 2013). There were no significant difference of disease duration across stages. Statistically significant differences were noted in the time of ventilator use, the time of tube feeding, and the time of complete quadriplegia among the 3 groups (Kruskal-Wallis). Multivariate analyses showed that the durations from onset to the time of ventilator use and complete quadriplegia had significant effecte on the progression from stage I to II, and that the duration from onset to the development of overt oculomotor limitation had signicant effect on the progression from stage IV to V.

Faster progression may predict the extent of communication impairment after ventilator use.Accurate prediction of communication impairment after ventilator use may promote medical and social preparation including early application of the brain-machine interface for future communication problems in ALS patients.

(Clin Neurol 2013;53:1396-1398)

Key words: amyotrophic lateral sclerosis, impairment of communication, stage of ALS focusing on the communication ability, predictors of communication impairment

＜シンポジウム　(4)-17-3 ＞ ALS におけるコミュニケーション障害とその対策：
完全閉じ込め状態への挑戦

Totally locked-in state 患者の脳と脊髄における障害部位と保全部位

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要旨：完全閉じ込め状態（TLS）の筋萎縮性側索硬化症（ALS）患者（ALS-TLS）と意思疎通をおこなうことを最終的な目的として，どのような感覚が ALS-TLS で保たれている可能性があるか，剖検例神経系の種々の感覚経路の保全 / 障害状態を神経病理学的に検索した．結果は，ALS-TLS では，視覚路，辺縁系（嗅覚路）などは保たれる傾向を示した．一方，体性感覚路，聴覚路，味覚路などは強く障害されていた．ALS-TLS 患者との意思疎通と，意思疎通のための BMI の開発と使用には，これらの所見を踏まえた入力方法を工夫する必要がある．

（臨床神経 2013;53:1399-1401）
Key words：筋萎縮性側索硬化症，完全閉じ込め状態，ブレインマシンインターフェース

はじめに

筋萎縮性側索硬化症（ALS）では，人工呼吸器装着症例で随意運動がまったく消失し，現在の補助手段では意思伝達不能（完全閉じこめ状態：Totally locked-in state; TLS）となる事がある．ALS 患者で TLS となった症例（ALS-TLS）に対し，私共をふくむ国内外の幾つかの研究機関がブレインマシーンインターフェース（BMI）により意思疎通を図る研究を開始した[1)~5)]．現時点では BMI は ALS-TLS 患者の意思を脳内の血流や脳波の変化から検出しようとする機器と方法であり，患者の脳内へ機器を埋め込んでその意思を検出する，あるいは患者へ外界の情報を伝達するための手段ではない．これに関連し，BMI が機能する（患者の意思［出力］の検出）ために，TLS-ALS 患者にどうやって周囲の人間の意向や質問を伝えるか（患者への入力），についての結論はえられておらず，かつ TLS-ALS 患者への入力経路，すなわち視覚経路，聴覚経路，皮膚感覚経路，味覚経路などの感覚路が脳内でどの程度障害され，あるいは保たれているか，についての報告はまったくみられない．ALS-TSL 患者との意思疎通を最終目的として，患者脳の感覚路の保全 / 障害状況を明らかにすることを目的とした．

方　法

人工呼吸器装着時まで認知症状がみとめられなかった日本人 ALS7 剖検例をもちいた．死亡時年齢 40 ～ 69 歳．男 4 症例，女 3 症例．孤発性は 4 症例で，家族性は 3 症例（家族性の内訳は SOD1 遺伝子変異 1 症例，FUS 遺伝子変異 2 症例である）．なお孤発例の 1 症例には SOD1 遺伝子変異があった．全例とも末期にはまったく意思疎通が不能で，林健太郎らの意思伝達障害の程度[6)]では Stage Ⅴ（Stage Ⅰ；文章にて意思表出が可能．Stage Ⅱ；単語のみ表出可能．Stage Ⅲ；yes/no のみ表出可能．Stage Ⅳ；残存する随意運動はあるが yes/no の確認が困難なことがある．Stage Ⅴ；全随意運動が消失して意思疎通不能な状態＝ TLS）であった．

これらの症例の脳重などの剖検時所見，およびホルマリン固定神経系パラフィン切片に HE，クリューバー－バレラ染色など，SOD（superoxide dismutase）1，FUS（fused in sarcoma），リン酸化神経細糸，シスタチン C などの免疫染色をおこなって神経病理学的に観察解析した．

結　果

ALS7 例の脳重は 715, 783, 1,019, 1,050, 1,170, 1,190, 1,233 g であった．いずれの症例でも脊髄前角細胞や脳幹の舌下神経核および顔面神経核などの下位運動ニューロンは著明に脱

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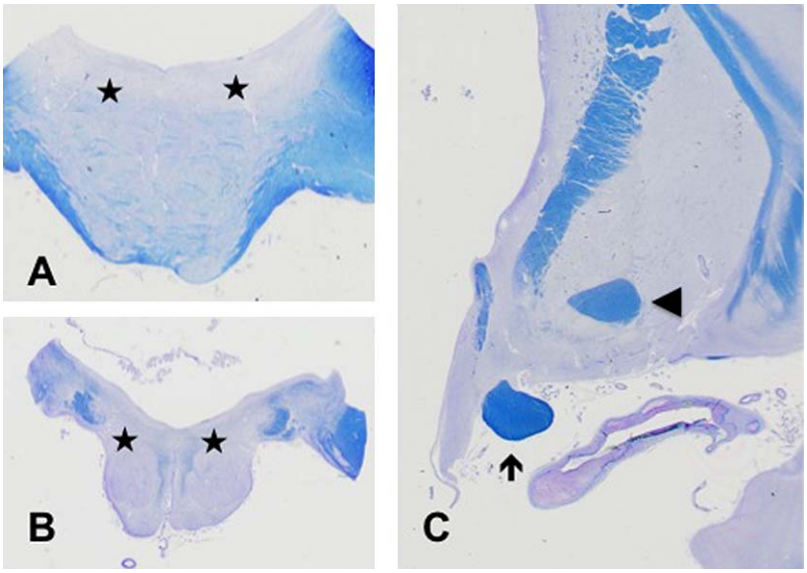

Fig. 1　完全閉じ込め状態 ALS.
A：完全閉じ込め状態 ALS の橋被蓋の萎縮（★）．B：完全閉じ込め状態 ALS の延髄被蓋の萎縮（★）．C：完全閉じ込め状態 ALS の視索（矢印）と前交連（矢頭）の保全．A ～ C：クリューバー－バレラ染色．

落し錐体路変性がみられた．また多くの症例で脳幹被蓋（Fig. 1A，B：★）や淡蒼球，視床が萎縮し，運動野ではベッツ細胞の脱落がみられた．715 g 脳は FUS 変異症例で大脳白質の萎縮が著明である．783 g 脳は「認知症をともなう ALS」がうたがわれた．これら以外の症例の大脳ではアンモン角をふくむ皮質の厚さ，白質の広さと色調は比較的良く保たれていた．SOD1 遺伝子変異 2 症例中 1 症例では SOD1 の沈着がみられた．すべての症例で保たれる傾向を示した解剖学的構造は，視神経，視索（Fig. 1C：矢印），外側膝状体，視放線，扁桃体，室傍核，視索上核，前交連（Fig. 1C：矢頭），マイネルト核などである．

考　察

ALS-TLS7 剖検症例の観察では，視覚路，辺縁系（嗅覚路）などが保たれる傾向を示した．しかし体性感覚路，聴覚路，味覚路などは強く障害されていた．これらの所見は，ALS-TLS 患者との意思疎通のための周囲からの入力手段として，視覚と嗅覚は可能性がありそうなこと，一方聴覚や触覚は不適当であることを示している．

過去に Hayashi ら[7]，Mizutani ら[8)9)]，Kato ら[10]，Nishihira ら[11]により報告されたように，ALS-TLS では脳幹被蓋すなわち網様体の強い障害が本研究でも全例で確認された．網様体の障害は賦活系の機能不全を示唆しており，外界からの刺激 / 情報が視覚中枢（鳥距野）や聴覚中枢（横回）まで達したとして，その後の情報処理が正確におこなわれて，脳波や脳血流の変化に反映しえるのか，という深刻な問題が存在する．ALS-TLS における BMI の開発と使用にはこれらの所見を踏まえ，一歩一歩着実な検討を積み重ねるべきであると考えられる．

結　論

ALS-TLS では，視覚路，辺縁系（嗅覚路）などは保たれる傾向を示した．一方，体性感覚路，聴覚路，味覚路ならびに脳幹被蓋，下位運動ニューロンなどは強く障害されていた．ALS-TLS 患者との意思疎通と，この為の BMI の開発と使用には，これらの所見を踏まえた入力方法を工夫する必要がある．

※本論文に関連し，開示すべき COI 状態にある企業，組織，団体はいずれも有りません．

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Abstract

Amyotrophic lateral sclerosis in totally locked-in state

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Seven autopsy patients with amyotrophic lateral sclerosis (ALS) in totally locked-in state (TLS) were examined neuropathologically. The patients were composed of 4 males and 3 females, and 3 with familial, 1 sporadic but with mutation in SOD1 gene, and 3 sporadic patients with unremarkable gene mutation. The brains weighed 715, 783, 1,019, 1,050, 1,170, 1,190 or 1,233 g. The tegmentum of the brain stem was markedly degenerated in every patient, and the tracts relating to the somatic sensory and auditory were involved in the lesions.

(Clin Neurol 2013;53:1399-1401)

Key words: amyotrophic lateral sclerosis (ALS), totally locked-in state (TLS), brain machine interface

＜シンポジウム　(4)-17-4 ＞ALS におけるコミュニケーション障害とその対策：
完全閉じ込め状態への挑戦

ALS 患者におけるコミュニケーション戦略：BMI の現状と展望

長谷川良平[1)]

要旨：産業技術総合研究所では重度運動機能障がい者のコミュニケーションを支援するために，認知型 BMI 技術をもちいた意思伝達装置「ニューロコミュニケーター」の開発を進めている．
本システムは，脳波のリアルタイム解析によってメッセージの候補（ピクトグラム）を同定することが可能である．このシステムを実現するために，3 つのコア技術，1）ポータブルかつワイヤレスの脳波計測機，2）高速・高精度の脳波解読アルゴリズム，3）階層的なメッセージ生成システムをもちいている．健常者実験では 1 回の選択あたり，95%以上の精度で予測をおこなうことができた（情報量として毎分 32 ビット相当）．現在，在宅患者対象のモニター実験を介してさらなる技術改良をおこなっている．

（臨床神経 2013;53:1402-1404）
Key words：脳波，意思伝達支援，ブレイン - マシン インターフェース

はじめに

筋萎縮性側索硬化症（Amyotrophic Lateral Sclerosis; ALS）を代表とする神経難病などに起因する運動機能障害によって不自由になった「生活の質」（Quality of Life; QOL）を向上させるために残存運動機能などを着目した「代替・拡大コミュニケーション」（Augmentative and Alternative Communication; AAC）に関する技術開発が世界的にも盛んになっていきている．簡単なものであれば，指差しが可能な患者用に各種のメッセージが描かれた絵カードや透明文字盤などがある．また，電子機器をもちいるものであれば，表情筋など身体の一部の動作を筋電センサや歪みセンサで検出し，ワンボタンスイッチにすることができる．そのばあい，単にナースコール的に利用することもできるし，パソコン上で動く専用ソフトウェアを操作して一文字ずつ入力するシステムも普及しつつある．

このような AAC の普及は，多くの意思伝達機能に障害を持つ人々の助けになっているものの，全身の運動機能が極度に低下した「完全閉じ込め状態（Totally Locked-in State; TLS）」の患者に対してはなすすべがないのが現状である．そこでわれわれの研究チームでは，脳と機械を直結するブレイン - マシン インターフェース（Brain-Machine Interface: BMI）技術[1)~4)]をもちいた意思伝達装置の研究開発に取り組んできた．その最初の成果として 2010 年 3 月に試作第 1 号機の開発に成功したのが，脳波 BMI による意思伝達装置「ニューロコミュニケーター」である（Fig. 1）[5)]．本論文では，その技術内容を紹介するとともに今後の課題に関して論じることとする．

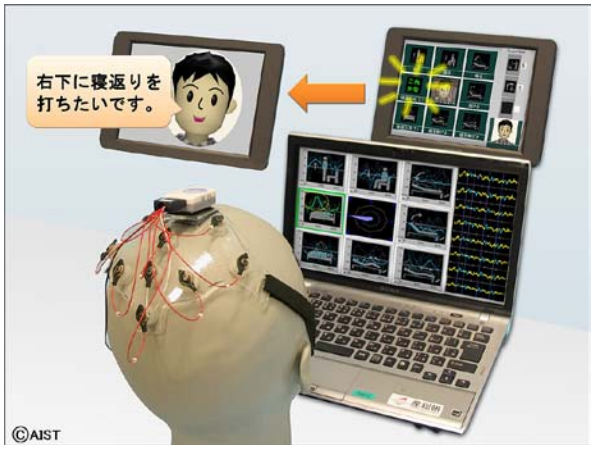

Fig. 1　脳波による意思伝達装置「ニューロコミュニケーター」．

脳波による意思伝達装置の開発

ニューロコミュニケーターは，外観から確認される装置としてはヘッドギアとノートパソコン，サブモニターの 3 つのパートから構成されており，それらは以下のような仕組みで働いている（Fig. 2）[6)]．まず，ユーザーが被るヘッドギアは軽量樹脂製であり，その前方には小型無線脳波計が搭載されている．脳波計からは複数のケーブルが伸びており，その先には金属製の電極が取り付けられている．頭部 8 ヵ所の電極はフジツボのような構造をしており，真ん中の小さな穴から導電性のジェルを流し入れて，髪の毛の間に染み込むことによって頭皮と電極がつながっている．これらの電極によって

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（受付日：2013 年 6 月 1 日）

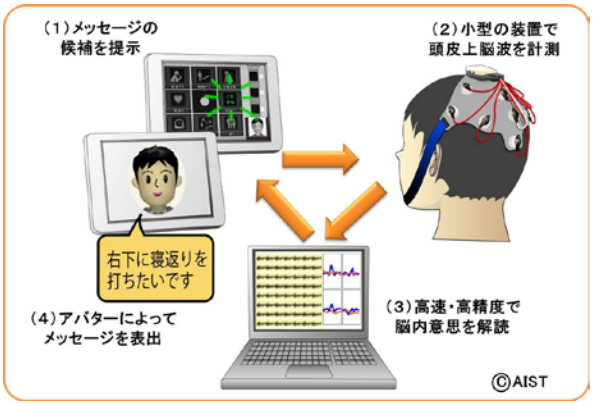

Fig. 2 「ニューロコミュニケーター」の動作原理.

計測した脳波データはリアルタイムでノートパソコンに無線送信されている. ヘッドギアをかぶったユーザーの眼前には, パソコンに接続された小さなサブモニターが置かれている. その画面上には伝えたいメッセージを表すような 8 種類のピクトグラム（単純な絵カードのようなアイコン）がメニューのように並んでいる. メニュー画面が提示されたのち, しばらくすると各ピクトグラムが順次, 疑似ランダムな順でフラッシュする（「これかな」という文字が一瞬表示）. この間, 注目しているピクトグラムがフラッシュしたときに, わずかではあるが脳波に一定の変化が生じる. この脳波の変化は, 専門用語で「P300」と呼ばれていて,「おやっ」と思うような注意の高まりがあったときに観察される [7].

脳波計から無線データを受け取ったノートパソコンでは, この P300 をリアルタイムで検出するプログラムが動いている. 各ピクトグラムに対するフラッシュが数回くりかえされた段階で, もっとも強い P300 を誘発したピクトグラムがユーザーの選びたいものであると推測する. P300 の検出には線形判別分析をベースとするパターン識別技術がもちいられており, 選ばれたメッセージは, パソコン画面上に現れたアバター（ユーザーの代わりとなる CG のキャラクター）が人工音声付きアニメーションによって周囲に伝えてくれる. なお, 本システムではメッセージを作るために 8 種類のピクトグラムから 1 つを選ぶ作業を 3 回くりかえしてメッセージを具体化していく方式により, 8 の 3 乗, つまり 512 種類という多様なメッセージを表現することができる.

体系的に実験をおこなった 10 名以上の健常者においては, 1 回の選択あたり（8 種類の選択肢に関して 5 回ずつのフラッシュをおこなう条件＝約 6 秒間）95％以上の精度で解読可能であった [8)9)]（情報量としては毎分約 32 ビットに相当）.

実用化に向けた課題

上述した技術のほとんどは, 2010 年 3 月のプレス発表までに基本的な開発を終わっており, 現在は実際の患者を対象としたモニター実験を実施中である [10]. 主に在宅療養中の患者宅を訪問し, 1 回あたり約 3〜4 時間の滞在時間のなか, 試作機の性能評価や患者側からのユーザビリティ評価に関する実証実験をおこなっている. 頭部の締め付け感の強い布製ヘッドキャップの代わりに樹脂製ヘッドギアを導入することにあったのも, このようなモニター実験での患者の意見がきっかけとなっている. 他にもモニター実験の進行とともに様々な改良がなされているが, 実際にはまだまだ苦戦を強いられている. 統制された環境の実験室において健常者を対象として実験するのであれば, かぎりなく 100％に近い精度で解読が可能であるにもかかわらず, 患者の生活する家庭内ではベッドサイドに医療機器や各種家電製品が存在し, 激しい電気的ノイズを発生するのである. また, 患者の容態も多種多様であり, 運動機能だけでなく認知機能もしくは意識レベルの低下もふくまれるケースなどもある. そのため, 現在は電気的ノイズに強いシステムの開発やルールのわかりやすいメッセージ生成方法の開発に重点を置いて実用化開発を加速している.

実用化に向けたさらなる改良のためにはひき続きモニター実験を継続する必要があるが, 今後は全国の医療機関や訪問介護ネットワークと連携関係を築き, 同時並行的に多数の臨床データを取得していきたいと考えている（連携先を募集中）. また, 試作機レベルとはいえ, 現バージョンのシステムを製品化することで, 意思伝達で困っておられる多くの患者とその家族に少しでも貢献できるように技術移転の作業も進めている.

※本論文に関連し, 開示すべき COI 状態にある企業, 組織, 団体はいずれも有りません.

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Abstract

Development of a cognitive BMI “neurocommunicator” as a communication aid of patients with severe motor deficits

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A cognitive brain-machine interface (BMI), “neurocommunicator” has been developed by the author’s research group in AIST in order to support communication of patients with severer motor deficits. The system can identify candidate messages (pictograms) in real time from electroencephalography (EEG) data, combining three core technologies; 1) a portable/wireless EEG recorder; 2) a high-speed and high-accuracy decoding algorithm; and 3) a hierarchical message generation system. The accuracy of the model at single predictions of the target was generally over 95%, corresponding to about 32 bits per minute for normal subjects. Monitor experiments have been also started for patients at their home, in which further technical improvements are required.

(Clin Neurol 2013;53:1402-1404)

Key words: EEG, communication aid, BMI

＜シンポジウム　(4)-17-5＞ALSにおけるコミュニケーション障害とその対策：
完全閉じ込め状態への挑戦

ALS患者におけるコミュニケーション戦略：脳外科からのアプローチ

吉峰　俊樹[1)]　柳沢　琢史[1)]　澤田　甚一[3)]
狭間　敬憲[3)]　望月　秀樹[2)]　平田　雅之[1)]

要旨：ALS患者の基本的コミュニケーション手段がすべて失われて「閉じ込め状態」となったばあい，脳活動そのものから患者の意思を読み取る方法が考えられる．これは脳信号の計測技術と解読技術（デコーディング）の発達により現実のものとなってきた．「ブレイン・マシン・インターフェイス（BMI）」と呼ばれる技術である．BMIのための脳信号をとらえる方法には非侵襲的方法と侵襲的方法があるが，本稿では，脳神経外科的アプローチとして私どもが研究，開発を進めている低侵襲的出力型BMIを紹介する．世界ではじめてALS患者において臨床研究がなされ，「考えるだけで」コミュニケーションツールを操作できた例である．

（臨床神経 2013;53:1405-1407）
Key words：ALS，コミュニケーション，BMI，閉じ込め症候群，皮質脳波

はじめに

Brain-machine interface（BMI）とは「脳と機械の間で直接信号をやりとりすることにより，失われた神経機能の代行あるいは回復促進に役立てる技術」である．BMIには脳信号を取り出して外部装置に伝える「出力型」と，外部機器で感知した信号を脳に伝える「入力型」があるが，下位運動ニューロンの脱落により全身の筋萎縮をきたした筋萎縮性側索硬化症患者における運動・コミュニケーション機能を支援するには，脊髄，末梢神経，筋などの本来の出力経路をもちいることなく，脳情報そのものを解読して直接，外部装置を操作することができる出力型BMIを利用する方法が考えられる[1)2)]．

出力型BMIは，解読する脳信号を取得する手段により，さらに「非侵襲的BMI」と「侵襲的BMI」に分けることができる．本稿では脳神経外科的アプローチとして研究，開発が進められている侵襲的BMI，とくに世界ではじめてALS患者において臨床研究がなされた私どもの硬膜下電極をもちいた低侵襲的出力型BMIを紹介する．

低侵襲的出力型BMIによる運動・コミュニケーションの支援

脳神経外科領域では，難治性てんかんや脳腫瘍患者において脳表に硬膜下電極をおいて大脳皮質機能マッピングをおこなうことがある．このような患者の承諾をえて，各種の運動課題遂行時の皮質脳波（ECoG）を解析した．その結果，運動開始時にはとくに高周波帯（γ波）のパワーが増加すること，そしてこのγ波の分布パターンが運動の種類ごとにことなることがわかる．この分布パターンの相違を機械学習（machine learning）の手法で解析すると，数種の運動の種類をほぼ正確にいい当てることができる[3)]．この方法をもちいると，実際に運動を行わなくてもイメージするだけでも運動の種類をいい当てることができるため，「考えただけで」モニター上のカーソル移動をおこなったりロボットアームの操作をおこなうことも可能であるため，これをALS患者の運動やコミュニケーションの支援にもちいることを計画した[4)]．

実際のALS患者への応用に先立ち，慢性の運動麻痺患者においても「運動をイメージするだけで」，その種類の解析が可能か否かを検討した．ALS患者では慢性の運動麻痺により運動皮質に機能低下がもたらされ，解析が困難な可能性も危惧されたからである．その結果，長期にわたる完全麻痺患者が運動をイメージしたばあい，運動麻痺のない患者や軽度の患者が実際に運動したばあいにくらべ，γ波の出現は不良ではあるが，解析は可能であることが明らかになった（Fig. 1）[5)]．またこの際，「運動をイメージすることが容易である」と自己申告した被験者では運動内容の正答率が高く，「イメージすることが困難である」と答えた被験者では低いことも明らかとなった．

以上の結果を踏まえ，私どもは倫理委員会の承認をえて，重症ALS患者に硬膜下電極をもちいたBMIの安全性と機能性を検討する臨床研究を開始した．被験者は，まず，MEGをもちいて運動イメージによる皮質運動野の脳信号の出現が良好であることを確認したのち，硬膜下電極を設置し，ECoGの解析による運動意図の推定が可能か否かを検討した．その結果，患者は「考えるだけで」コンピュータ（オペレート・

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（受付日：2013年6月1日）

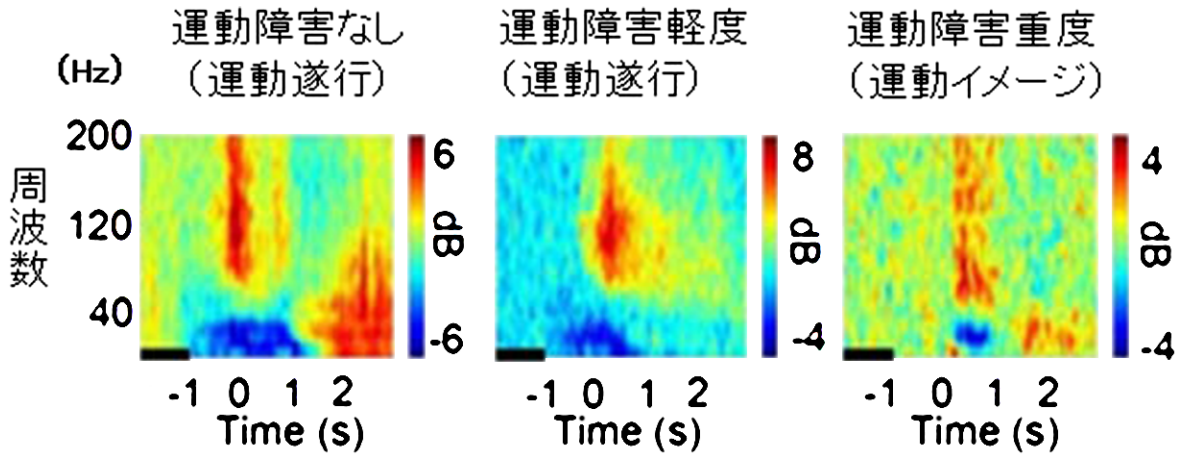

Fig. 1　Power spectra of movement-related ECoG of patients with no (left), mild (center) and severe (right) paresis. Gamma band power decreases as the paresis increases. Patient with severe paresis only imaged to move the hand.

Fig. 2　An ALS patient with severe motor disability after implantation of subdural electrodes (left). Using our BMI system, he operated OPERATENAVI only by "thinking".

ナビ）を操作して文章を綴ることが可能であった（Fig. 2）.

おわりに

重症ALS患者が呼吸不全に陥った際の生命維持については，なお国際的議論が続いている状況であるが，本邦では，呼吸麻痺出現時に長期機械呼吸（気管切開をともなう人工呼吸）を選択する率が24.5%（厚労省班会議）～46%（日本ALS協会）である[6]．長期機械呼吸の選択が例外的といわれる米国などとくらべ，世界的に突出した値である．しかし，最近の国際的調査では，重症ALS患者であっても患者自身が自己評価したQOLは意外に良好であると報告され[7)8)]，長期機械呼吸は患者の選択肢として見直される傾向もある．

患者自身の生活満足度が予想外に高いとしても，生活の多くの面で不自由を被っていることには変わりなく，とくに患者はコミュニケーション能力の喪失を強く悲嘆している．私どもが日本ALS協会の協力をえて，患者ないしその家族がBMIに期待する機能を調査した結果でも，コミュニケーション手段としての機能を「強く希望」ないし「希望」していた[9]．川口によれば，呼吸麻痺時には長期機械呼吸を希望した患者であっても，眼球運動まで喪失して閉じ込め症候群にいたった際には生命維持を中止して欲しい胸希望していたた患者もあったという[10]．人の生存基盤のひとつとして，コミュニケーション機能には筆舌に尽くしがたい重要性があるものと想像される．BMIはその支援手段としておそらくもっとも実現性の高い技術であると考えられる．

※本論文に関連し，開示すべきCOI状態にある企業，組織，団体はいずれも有りません．

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Abstract

Communication with ALS patients: Neurosurgical approach

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By progression of the disease, motor neurons degenerate in patients with amyotrophic lateral sclerosis (ALS) eventually lose nearly all voluntary muscles in the body. They are awake and aware but cannot move or communicate (locked-in state). Since the function of the brain is preserved, one possible measure to support their communication is to interpret their motor intention by decoding (deciphering) brain signals and present it with external devices. This technology called “brain-machine interface (BMI)” is now close to clinical use in Japan and USA.

In our system, we record electrocorticogram (ECoG) obtained with subudural electrodes during their motor imagery, decode it and determine the movement they intended. So far, one patient of ALS with severe paralysis, implanted with this electrodes, successfully operated the PC communication tool only by thinking.

(Clin Neurol 2013;53:1405-1407)

Key words: ALS, communication, BMI, locked-in, ECoG

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Predictors of impaired communication in amyotrophic lateral sclerosis patients with tracheostomy-invasive ventilation

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Abstract

Predictors of communication impairment in patients with amyotrophic lateral sclerosis (ALS) using tracheostomy-invasive ventilation (TIV) were investigated. Seventy-six ALS patients using TIV were enrolled and classified into three subgroups of communication ability: patients who could communicate with communication devices (Stage I), patients who had difficulty with communication (Stage II, III, or IV), and patients who could not communicate by any means (Stage V). Predictors of communication impairment were analysed by the Cox proportional hazard model.

Results demonstrated that there were no significant differences in disease duration between subgroups. Within 24 months after disease onset, patients who needed TIV and tube feeding, developed oculomotor impairment or became totally quadriplegic and progressed from Stage I to II and V significantly earlier. Multivariate analyses revealed that within 24 months from onset, the need for TIV and progression to total quadriplegia were significant events in patients who progressed to Stage II, whereas the development of oculomotor limitation was significant in patients who progressed to Stage V. In conclusion, TIV, impaired oculomotor movement and total quadriplegia are predictors of severe communication impairment. Rapid disease progression might indicate future communication impairment after the use of TIV. We highly recommend early detection of impaired communication and identification of the best methods of communication.

Key words: *Amyotrophic lateral sclerosis, ventilation, communication ability stage, predictors, communication impairment, totally locked-in state*

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of upper and lower motor neurons. Impaired speech is caused by bulbar or pseudobulbar palsy. Non-verbal communication may also be affected due to quadriplegia and respiratory compromise. ALS ultimately results in respiratory failure and death, unless treated with mechanical ventilation. Use of non-invasive ventilation (NIV) may improve survival temporarily (1). However, tracheostomy-invasive ventilation (TIV) supports breathing and may extend survival for years, well beyond respiratory failure in patients with ALS (2). Therefore, TIV is referred to as long-term mechanical ventilation (LTMV). Nevertheless, ALS patients using TIV can achieve a reasonable quality of life (QoL) and are often grateful to be alive (3). Maintaining an effective method of communication is a primary requirement for achieving a reasonable QoL in ALS patients using LTMV (4). In contrast, poor communication decreases QoL under the same circumstances. Difficulty in understanding patients with impaired communication may also result in the inability to accurately assess their complaints and identify appropriate care interventions, leading to delay in the effective management of care (5).

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A study of the clinical profiles of Japanese patients with sporadic ALS has shown a higher percentage (29.3%) using TIV compared with ALS patients in North America or Europe (2.1–5.4%) (6). Use of an appropriate augmentative and alternative communication (AAC) system is essential for achieving and maintaining effective communication (7). AAC includes all forms of communication (other than oral speech) that are used to express thoughts, needs, wants, and ideas. Patients with ALS who have severe speech impairment rely on AAC to supplement existing speech or replace speech that is not functional. Approximately 13% of ALS patients with TIV develop progressive immobility (8) and a totally locked-in state (TLS) (9,10), which refers to a state in which all voluntary movements are lost and communication is impossible by any means. Recently, various AAC devices and brain-machine interfaces (BMIs) have been developed and used by ALS patients with severe communication impairment in an attempt to communicate (7,11,12). Previously, we determined that the use of an electroencephalography based BMI system was difficult for patients with severe communication impairment (13).

In a previous study, we proposed a classification of communication ability ranging from Stage I to Stage V, and we analysed the clinical characteristics of patients with ALS (14). Furthermore, the clinical analysis of 29 patients with autopsy-confirmed ALS revealed that those individuals who had reached Stage V had begun to use TIV significantly earlier than those patients classified with Stage IV or less in their final assessment, and they frequently had a family history of ALS. The aim of this study was to establish the predictors of communication impairment in ALS patients with TIV by analysing the relationship between specific clinical findings and the prognosis for communication impairment.

Subjects and methods

Seventy-six patients with ALS who were using TIV were retrospectively studied. Patients were selected from two populations. The first group included 29 patients among the 132 in our hospital with autopsy-confirmed ALS during the period 1980–2011; they were examined previously and had survived on TIV without overt dementia, anoxic encephalopathy, or any other neurological complications (14). The second group included 53 patients with ALS on TIV who had been continuously followed at our hospital from 2005 to 2012. Of the 53 patients, we excluded two with overt dementia that appeared at the initial stages of ALS, and four patients with concomitant Parkinsonism of unknown cause. Finally, 47 patients were enrolled from the second group. All the enrolled patients showed relentless progressive courses both before and after ventilator use, and no contributory diseases other than ALS were found during follow-up. Among the second group of 47 patients, seven died and were not autopsied, and three dropped out of the study at Stages I, II and V, respectively, over a period of five years. Thus, 37 survivors among the 47 patients remained at the end of March 2012.

The patients' neurological status and their ability to communicate were investigated based on a review of medical and nursing records. Among 76 patients enrolled, there were none with overt dementia on routine neurological examination, although full investigations for cognitive and psychiatric function were not performed in all patients. All patients showed normal orientation, reasonable conversation content, and satisfactory decision-making. After administration of TIV, many of the patients used a communication board and showed reasonable communication ability until occurrence of severe ophthalmoplegia, although a precise evaluation of cognitive function was not possible in late-stage patients. The ability to communicate with any AAC devices was assessed as previously reported in patients from each of the stages (I–V) as follows (Table I): Stage I, communicated using sentences; Stage II, communicated using one word answers only; Stage III, only communicated using non-verbal yes/no responses; Stage IV, only communicated occasionally with uncertain non-verbal yes/no responses; Stage V: unable to communicate by any means (14). At present, Stage V is indicative of TLS (9,10). When a patient's ability to communicate fluctuated, the stage of communication ability at the time when their communication remained stable was determined and used.

For clinical assessments, we evaluated the following factors: gender, age at ALS onset, affected body region at onset, family history of ALS, genetic abnormalities upon examination, and disease duration. The time from ALS onset to the following four clinical events was determined: need for TIV, feeding tube placement, development of overt oculomotor limitation, and progression of immobility to total quadriplegia. The overt oculomotor limitation was defined as constant abnormal ocular movements during a bedside neurological examination by at least two neurologists. The evaluation for ocular movements at the bedside included examinations of slow pursuit movement and ocular saccade speed in the vertical and horizontal gaze. The abnormalities usually corresponded with severe vertical ophthalmoparesis and slow saccade (slow eye movement).

Table I. A classification of ALS clinical stages focusing on the communication ability.

Stage I	communicates using sentences
Stage II	communicates using one word answers only
Stage III	only communicates using non-verbal yes/no responses
Stage IV	only communicates occasionally with uncertain non-verbal yes/no responses
Stage V	unable to communicate by any means

Revised from Table I in reference 14.

Total quadriplegia indicated that even slight muscle movements were no longer possible.

Statistical analysis

Patients were classified into three subgroups according to their stage of communication ability just before death or at the end of the study. Group 1 consisted of patients who could communicate with AAC (Stage I). Group 2 consisted of patients who had difficulty with communication (Stage II, III, or IV). Group 3 consisted of patients who could not communicate by any means (Stage V). Data comparisons among the three groups were performed using the Kruskal-Wallis test or χ^2 test.

The analyses for the progression of communication impairment were performed by reclassifying the patients into two subgroups according to the presence or absence of the aforementioned clinical events 24 months after ALS onset: A) need for TIV; B) need for feeding tube; C) overt oculomotor limitation; and D) total quadriplegia. The rates of attenuated progression for communication ability from Stage I to II and to Stage V were assessed for the four clinical events (A–D) using the Kaplan-Meier method of analysis and the log-rank test.

Finally, univariate and multivariate analyses were performed for progression from Stage I to II and to Stage V using the Cox proportional hazard model to elucidate the predictors for progression of the stage of communication ability, adjusting for age at onset, affected body region at onset, familial or genetic abnormalities, and the four clinical events discussed previously.

All analyses were performed using Predictive Analytics Software (PASW) Statistics version 21 (IBM SPSS Statics) and p-values $<.05$ were considered significant for all the statistical analyses.

This study was approved by the ethics committees of Tokyo Metropolitan Neurological Hospital and Tokyo Metropolitan Institute of Medical Science.

Results

For all 76 patients, the median age at onset was 54.0 years (interquartile range (IQR) 44.3–63.8 years) and median disease duration was 104.0 months (IQR 61.5–169.3 months). The clinical characteristics of each subgroup are described in Table II and Figure 1. Among subgroups, there were no significant differences in gender, age at onset, affected body region at onset, total disease duration from onset and TIV duration. Figure 1 shows that there were many patients whose communication ability was well preserved even after very long-term ventilator use. There were, however, significant differences in the times from onset to the need for TIV, the need for a feeding tube, and progression to total quadriplegia; these times were shortest for group 3 (Table II). There were no statistically significant differences for other clinical events among subgroups.

The Kaplan-Meier analysis and log-rank test (Figures 2, 3) showed that for the clinical events assessed 24 months after onset, the subgroup of patients who experienced them progressed to both Stage II and V significantly earlier than the subgroup of patients for which the clinical events were absent within 24 months after onset.

Using the Cox proportional hazard model, univariate analyses for the progression from Stage I to II within 24 months after onset showed that age at

Table II. Clinical characteristics based on stage of communication ability.

	Group 1 (Stage I)	Group 2 (Stages II-IV)	Group 3 (Stage V)	p-value*
Patients, *n* (%)	36 (47.3)	27 (35.5)	13 (17.2)	
Males, *n* (%)	23 (63.9)	19 (70.4)	6 (46.2)	.328†
Median age at onset, years (IQR)	54.5 (47.3–61.0)	56.0 (49.0–68.0)	52.0 (38.5–60.5)	.187‡
Bulbar-onset patients, *n* (%)	7 (19.4)	7 (25.9)	1 (7.7)	.397†
Clinical event, *n* (%)				
Familial ALS or sporadic ALS with genetic abnormalities	3 (8.3)	2 (7.4)	4 (30.7)	.067†
Feeding tube	32 (88.9)	27 (100)	13 (100)	.096†
Overt oculomotor limitation	9 (25.0)	25 (92.6)	13 (100)	<.0001†
Total quadriplegia	15 (41.7)	21 (77.8)	13 (100)	<.0001†
Median disease duration, months (IQR)	104.0 (59.3–168.8)	86.0 (57.0–199.0)	126.0 (83.5–171.0)	.585‡
Median TIV duration, months (IQR)	54.0 (22.0–76.75)	66.0 (38.0–154.0)	102 (67.5–138.0)	.061‡
Median time from onset to clinical event, months (IQR)				
Need for TIV	56.5 (29.8–82.0)	21.0 (17.0–36.0)	15.0 (12.5–25.5)	<.0001‡
Need for feeding tube	56.0 (29.8–93.5)	25.0 (15.0–61.0)	16.0 (12.0–29.0)	.001‡
Development of overt oculomotor limitation	84.0 (46.0–147.0)	50.0 (29.5–115.0)	33.0 (16.0–69.0)	.107‡
Progression to total quadriplegia	78.0 (50.0–183.0)	65.0 (43.5–126.0)	31.0 (22.0–55.5)	.002‡

ALS: amyotrophic lateral sclerosis; IQR: interquartile range; TIV: tracheostomy- invasive ventilation; Group 1 = patients who could communicate with augmentative and alternative communication (AAC Stage I); Group 2 = patients who had difficulty with communication (Stage II, III, or IV); Group 3 = patients who could not communicate by any means (Stage V). *p-value <.05 is significant.
†$\chi 2$ test.
‡Kruskal-Wallis test.

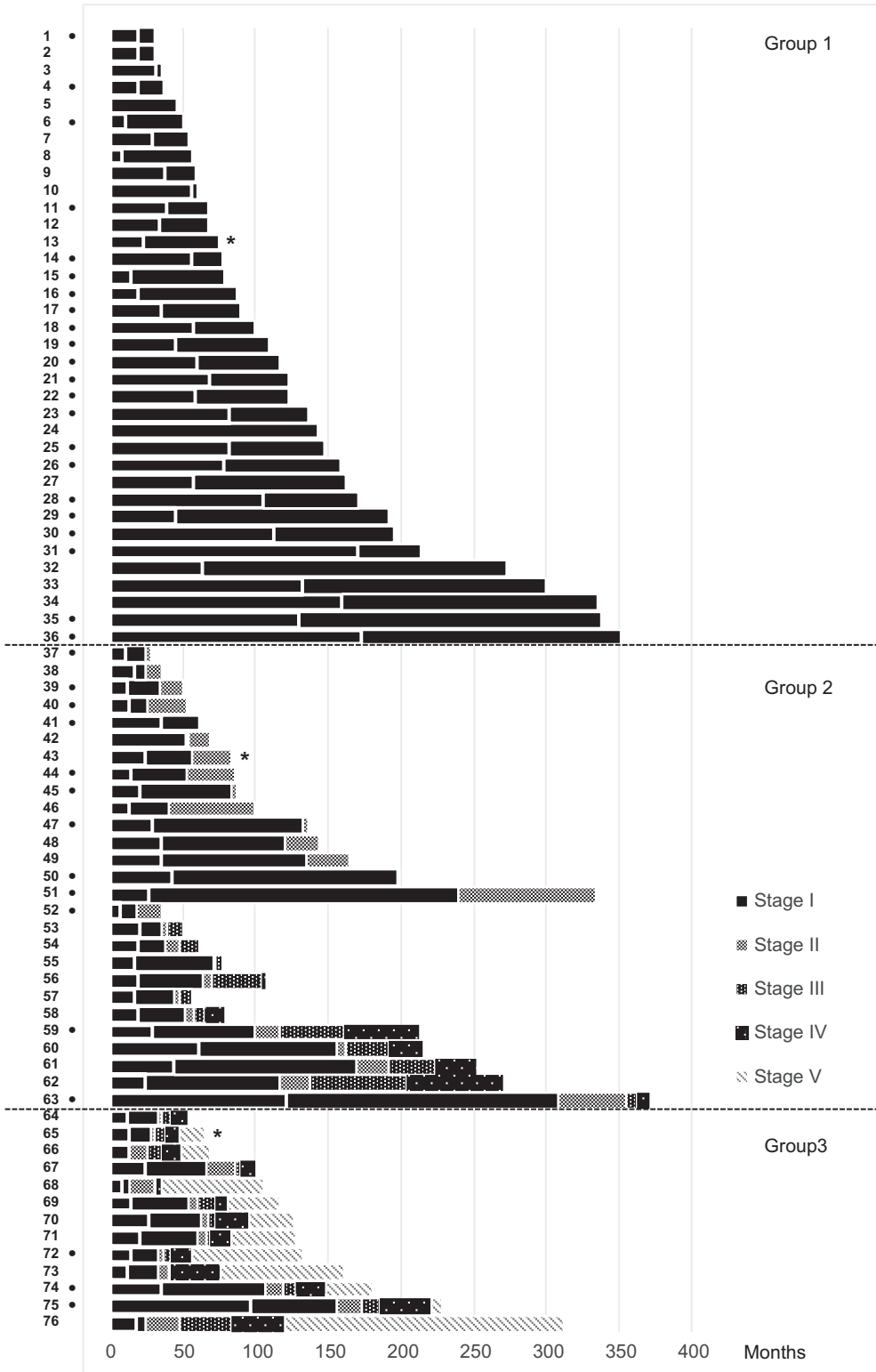

Figure 1. Disease duration and communication stage of 76 patients with ALS. Each stage of communication impairment is shown by a different grey colour. White lines in each column represent the initiation time of TIV. Closed circles represent patients who were alive at the end of March 2012, and asterisks represent patients who dropped out of follow-up.

onset, need for TIV, need for a feeding tube, development of overt oculomotor limitation and progression to total quadriplegia were significant. Multivariate analyses for the progression from Stage I to II within 24 months after onset showed that the need for TIV and for development of total quadriplegia were significant (Table III). For progression to Stage V within 24 months after onset (Table IV), the univariate analyses showed that progression in the stage of communication ability was significantly associated with the need for TIV, the need for a feeding tube, development of overt oculomotor limitation, and the progression to total quadriplegia. The multivariate analyses showed that the development of overt

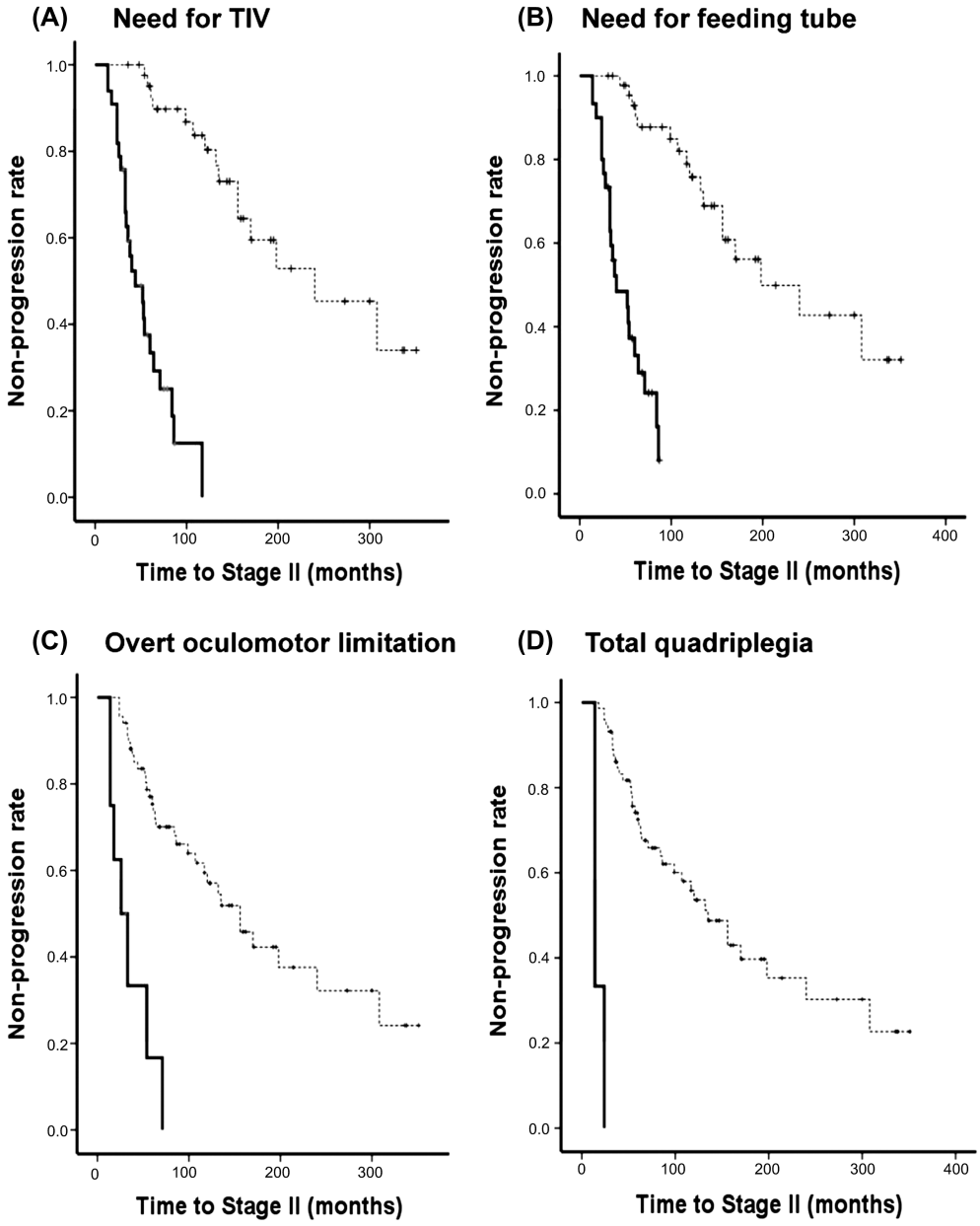

Figure 2. Kaplan-Meier curves showing the rate of attenuated progression to Stage II communication ability in patients with ALS. Kaplan-Meier curves of the rate of non-progression to Stage II for 76 patients based on the presence (bold line) and absence (dotted line) of clinical events (A–D) 24 months after disease onset. + shows censored patients.

oculomotor limitation within 24 months after onset had a significant effect on the progression to Stage V.

Discussion

This study showed that a faster rate of progression in the early phase of ALS predicted the rate of impaired communication as the disease advanced. The patients who reached Stage V not only began TIV earlier (14) but also had a feeding tube and progressed to total quadriplegia more rapidly than patients who did not reach Stage V. Since our goal was to determine the predictors of impaired communication in the early phase of ALS, we focused on specific clinical events within 24 months after ALS onset. The use of TIV, need for a feeding tube, signs of overt oculomotor limitation and total quadriplegia, indicated by early progression of respiratory dysfunction, bulbar palsy, oculomotor limitation and limb disability, are strongly related to progression of impaired communication.

Previous epidemiological studies have shown several factors related to shorter survival in ALS: older age (6,15,16), bulbar onset (15,16,17), a reduction in forced vital capacity (16), a rapid reduction in body mass index (18,19), a rapid decrease in the revised ALS Functional Rating Scale (ALSFRS-R) score (16,20), and psychosocial factors (21). A high ALSFRS-R score at TIV initiation also predicts a long-term survival after TIV (22). These reported prognostic factors were for survival of ALS; thus, the endpoints of studies are usually death or the need for TIV (6,15–22). However, while TIV prolongs

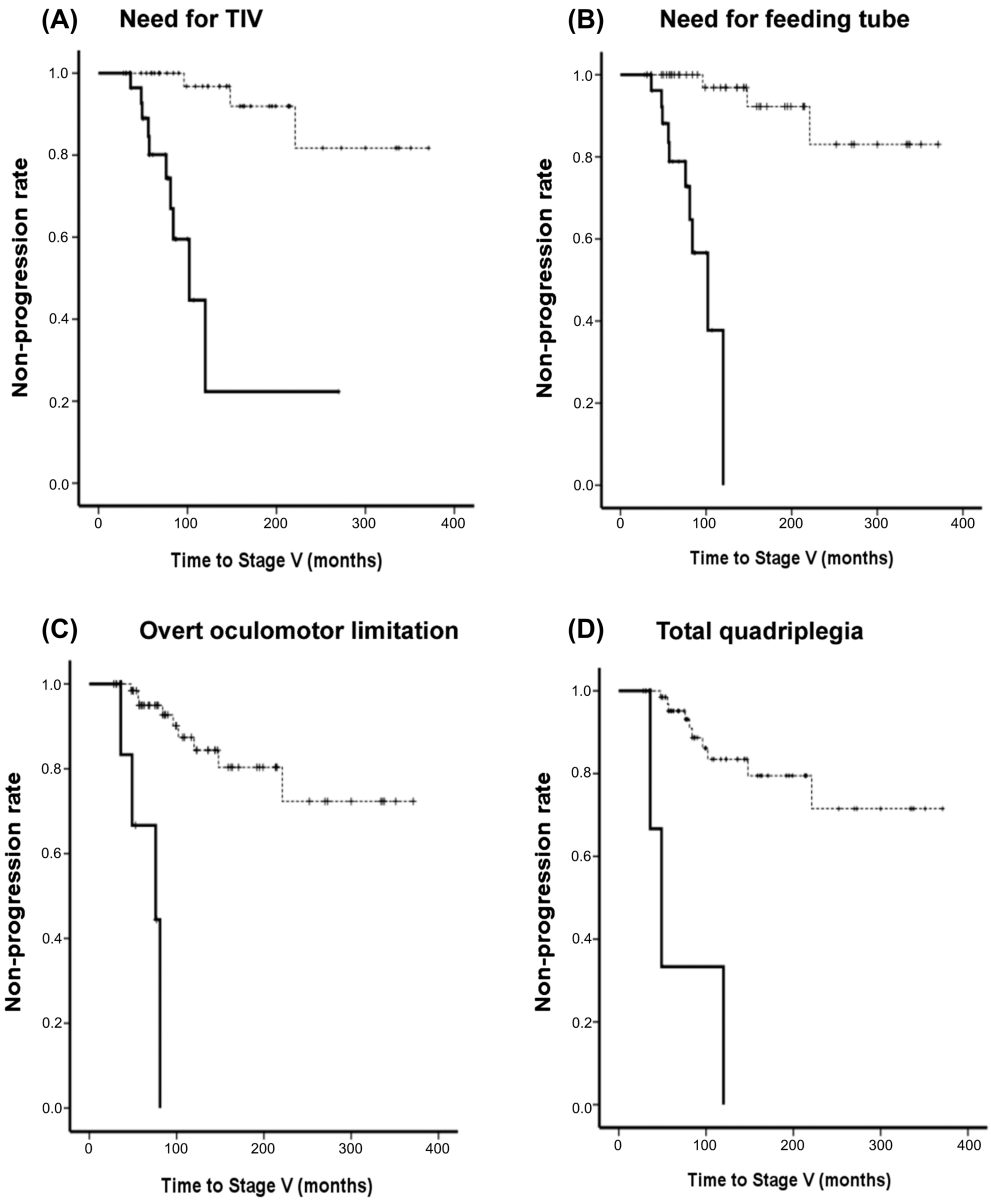

Figure 3. Kaplan-Meier curves showing the rate of attenuated progression to Stage V communication ability in patients with ALS. Kaplan-Meier curves of the rate of non-progression to Stage V for 76 patients based on the presence (bold line) and absence (dotted line) of clinical events (A–D) 24 months after disease onset. + shows censored patients.

survival, the disease progression never stops and often shows a wide and multisystem distribution of neurodegenerative lesions spreading to areas other than the motor system (9). Our patients with TIV showed much longer survival lengths than previously reported (Table II) (23–28), and we needed predictors not only for survival but also to aid in the maintenance of care interventions. Seriously disabled patients with TIV cannot be evaluated even with the extended version of ALSFRS-R (29). Thus, our new classification of clinical stages focusing on the degree of communication impairment is useful for such advanced patients with ALS.

In patients with ALS using TIV, TLS is referred to as the most severe state of ALS, especially due to the inability to communicate by any means (9,10). However, only 17.2% of our patients in this study developed TLS, which is a little higher than the 13% of patients previously reported in Japan (8). In other words, about 80% of ALS patients using TIV can communicate with appropriate devices for AAC. It is very important to carefully assess ALS patients using TIV and develop effective methods for maintaining communication. Eye movement is the most important communication tool in such patients. Oculomotor limitation was a predictor for developing Stage V in this study, and about 30% of patients using TIV for more than nine years were reported to show ophthalmoplegia (6), indicating the importance of the oculomotor evaluation. The oculomotor limitation in ALS patients with TIV is initially with a supranuclear mechanism (30–32), but in the advanced stage of TLS the brainstem oculomotor nucleus is also involved neuropathologically (9,33), and is the focus

Table III. Univariate and multivariate analyses for progression to Stage II by Cox proportional hazard model.

		Hazard ratio	95% CI	*p*-value*
Univariate analysis				
Gender	males vs. females	1.236	0.650, 2.350	.517
Age at onset	younger vs. older than 65 years	1.039	1.007, 1.073	.015
Affected body region at onset	non-bulbar vs. bulbar	1.139	0.521, 2.491	.745
Familial or genetic abnormalities	non-familial or no genetic abnormalities vs. familial or genetic abnormalities	1.410	0.590, 3.372	.440
Time from onset to clinical event				
Need for tracheostomy-invasive ventilation	shorter vs. longer than 24 months	17.006	6.445, 44.876	<.0001
Need for feeding tube	shorter vs. longer than 24 months	15.257	5.630, 41.344	<.0001
Development of overt oculomotor limitation	shorter vs. longer than 24 months	7.959	3.273, 19.357	<.0001
Progression to total quadriplegia	shorter vs. longer than 24 months	50.669	9.711, 264.369	<.0001
Multivariate analysis				
Age at onset	younger vs. older than 65 years	1.028	0.993, 1.065	.116
Time from onset to clinical event				
Need for tracheostomy-invasive ventilation	shorter vs. longer than 24 months	7.762	1.660, 36.297	.009
Need for feeding tube	shorter vs. longer than 24 months	1.871	0.434, 8.062	.401
Development of overt oculomotor limitation	shorter vs. longer than 24 months	1.904	0.721, 5.030	.194
Progression to total quadriplegia	shorter vs. longer than 24 months	67.087	6.634, 707.201	<.0001

CI: confidence interval
*$p < .05$ is significant.

of the phosphorylated 43-kDa TAR DNA-binding protein (pTDP-43) pathology (34,35).

The comparison between subgroups with Stages I, II–IV and V, however, showed no significant differences in the total disease durations (Table II, Figure 1). This suggests that there might be no continuity in the disease course between Stages I and V, i.e. not all patients develop into TLS, and some populations can remain in Stage I even with long-term use of a ventilator. Our findings should be useful for those making decisions about TIV initiation.

One of the limitations of this study is that a gene search was not conducted in all cases. The univariate analysis showed a non-significant effect of gene mutations or a family history of ALS on progression to Stage V (Table IV; $p = 0.051$) as suggested by a previous preliminary study (14). Further study is required to elucidate which type of gene abnormalities would cause severe communication impairment. The other limitation is the lack of full evaluations of cognitive function. Although we excluded the patients with overt dementia at the early stages, severe communication impairment at the advanced

Table IV. Univariate and multivariate analyses for progression to Stage V by Cox proportional hazard model.

		Hazard ratio	95% CI	*p*-value
Univariate analysis				
Gender	males vs. females	2.180	0.730, 6.507	.163
Age at onset age	younger vs. older than 65 years	0.993	0.949, 1.040	.777
Onset body region	non-bulbar vs. bulbar	0.378	0.049, 2.939	.353
Familial or genetic abnormalities	non-familial or no genetic abnormalities vs. familial or genetic abnormalities	3.266	0.996, 10.706	.051
Time from onset to clinical event				
Need for tracheostomy-invasive ventilation	shorter vs. longer than 24 months	15.631	3.934, 62.108	<.0001
Need for feeding tube	shorter vs. longer than 24 months	53.267	6.121, 463.532	<.0001
Development of overt oculomotor limitation	shorter vs. longer than 24 months	22.473	4.866, 103.785	<.0001
Progression to total quadriplegia	shorter vs. longer than 24 months	13.821	3.629, 52.633	<.0001
Multivariate analysis				
Familial or genetic abnormalities	non-familial or no genetic abnormalities vs. familial or genetic abnormalities	1.344	0.239, 7.565	.737
Time from onset to clinical event				
Need for tracheostomy-invasive ventilation	shorter vs. longer than 24 months	0.706	0.013, 37.528	.863
Need for feeding tube	shorter vs. longer than 24 months	38.403	0.450, 3274.486	.108
Development of overt oculomotor limitation	shorter vs. longer than 24 months	7.034	1.323, 37.393	.022
Progression to total quadriplegia	shorter vs. longer than 24 months	1.964	0.331, 11.648	.457

CI: confidence interval.
*$p < .05$ is significant.

stages might have masked the underlying dementia. A considerable number of patients in Stage V show severe brain atrophy on neuroimaging examinations (unpublished data), suggesting an underlying cognitive decline in Stage V that could not be detected because of the severe motor disability.

In conclusion, based on our findings of potential prognostic factors for progression to higher stages of communication impairment, we highly recommend early detection of any impairment of communication and identification of optimal methods of communication for achieving the best possible QoL.

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Sympathetic Hyperactivity and Sympathovagal Imbalance in Amyotrophic Lateral Sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder with progressive loss of upper and lower motor neurons. Autonomic nervous abnormalities, including sympathetic hyperactivity and sympathovagal imbalance, have been found in both early and advanced stages of ALS. In early stage, the dysfunction may be subclinical. Occasionally, elevated blood pressure or heart rate and increased sweating may be observed. In advanced stage when ventilators are required, the sympathetic hyperactivity may lead to hypertensive crisis without counter-regulation of heart rate, followed by the consecutive circulatory collapse, known as the 'autonomic storm'. The symptoms of 'autonomic storm' are similar to that of 'baroreflex failure', and 'autonomic storm' indicates poor prognosis and may result in sudden death. Careful evaluation and individual treatment are strongly suggested, although appropriate therapeutic approaches have not been established. Causative central nervous lesions remain to be elucidated, although the limbic system may be involved. The autonomic dysfunction further supports the concept that ALS is a multisystem-degenerative disease.

Keywords

Amyotrophic lateral sclerosis, autonomic nervous system, sympathetic hyperactivity, sympathovagal imbalance, baroreflex

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder with selective and progressive loss of upper and lower motor neurons. Although this disease was identified more than 140 years ago by Charcot, the pathogenesis has yet remained unknown. Recent advancements in neuroimaging, neurophysiology and neuropathology have elucidated subclinical or clinical involvements of the non-motor systems, in addition to the upper and lower motor neurons. Accumulating evidence shows that autonomic nervous abnormalities are found in both early and advanced stages of ALS.[1] The autonomic dysfunction may be clinically evident and devastating, especially in advanced stage when ventilators are required. Here, the autonomic dysfunctions in ALS are reviewed.

Clinical and Haemodynamic Studies

The autonomic nervous system is divided into sympathetic and parasympathetic divisions, each with a central and peripheral component. The central component is known as the central autonomic network and regulates the balance between the sympathetic and parasympathetic regulations of the visceral organs. The term 'autonomic failure' is mainly used to describe impairment of the sympathetic vasomotor efferent systems, including the intermediolateral nucleus (IML) of the spinal cord and sympathetic ganglion. Autonomic failure represents orthostatic or postprandial hypotension or syncope attacks caused by reduced sympathetic tone, which are often observed in multiple system atrophy, but patients with ALS rarely develop such symptoms. Contrary, cardinal autonomic dysfunctions in ALS are sympathetic hyperactivity and sympathovagal imbalance, the clinical manifestations of which often resemble the symptoms of autonomic overactivity or baroreflex failure.[2,3] Differential diagnosis between autonomic failure and baroreflex failure should be carefully performed, since the therapeutic approaches for these two are quite different.[3]

In early stage of ALS, the sympathetic hyperactivity may be subclinical. Patients with ALS do not usually show overt hypertension needing treatment. However, some patients, especially, the ones with bulbar type of ALS experience palpitation, facial flushness and hot sensation around the face. These symptoms might be caused by increased sympathetic tone. Haemodynamic studies have shown that compared to healthy subjects, patients in early stage of ALS have higher resting heart rate and blood pressure.[4–7] Plasma norepinephrine level was also reported to be elevated and not correlated with the extent of motor disability.[8–12] Since plasma norepinephrine level reflects the amount of norepinephrine released from the peripheral sympathetic nerve terminals, its elevation is an indirect indicator of increased sympathetic tone. Although intravenous norepinephrine infusion test has shown preserved adrenoceptor function in the peripheral blood vessels, some patients on ventilators exhibit blunted response to infused norepinephrine, indicating down-regulated hyposensitivity of peripheral adrenoceptors induced by constantly high level of plasma norepinephrine.[13]

Muscle Sympathetic Nerve Activity

Direct measurement of sympathetic activity is possible as muscle sympathetic nerve activity (MSNA) by recording postganglionic nervous impulses in peripheral nerves, using the microneurographic recording technique. MSNA is considered to reflect sympathetic activity related to cardiovascular control of vascular resistance. Many investigators have reported that MSNA was increased in patients in early stage of ALS.[5,14–17] MSNA was elevated in ALS when quantified as the number of sympathetic burst (bursts/min or bursts/100 beats).[14,16] Response of MSNA following head-up tilt, however, is less in patients with ALS than in control subjects.[17] Similarly, various stimulation techniques such as Valsalva manoeuvre, apnoeic stimulation, painful stimulation, cold

water immersion, glucose ingestion and lower body negative pressure, showed blunted responses of MSNA in patients having ALS.[5,14] These reduced responses were attributed to the 'ceiling effect' caused by the constantly elevated sympathetic tone at resting state.[14] The level of MSNA in ALS did not correlate with motor disability, respiratory function, or disease duration, suggesting a primary phenomenon as well as motor neuron degeneration. Although Shindo et al. reported that MSNA level gradually decreased with disease progression in individual patients,[15] chronological variation of MSNA remains in argument.[14] In addition, the chronological improvement of MSNA is inconsistent with the markedly increased sympathetic tone and autonomic storm in patients at the advanced stage of ALS when ventilators are required.[10]

Variability of Heart Rate and Blood Pressure

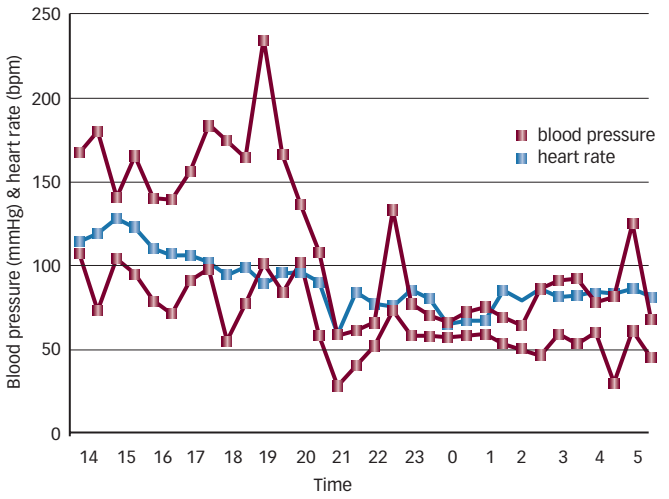
Another effective technique to measure the sympathetic and parasympathetic function is the time and frequency domain analysis of heart rate and blood pressure, especially the power spectrum analysis. Heart rate variability (HRV) in the low-frequency (LF) band (0.04–0.15 Hz) is mediated by both sympathetic and parasympathetic influences, whereas oscillation in the high-frequency (HF) band (0.15–0.40 Hz) is derived from cardiovagal modulation.[18] A combination of HRV and blood pressure variability analysis can indicate not only baroreflex sensitivity (for the LF band), but also functioning of cardiorespiratory transfer (for the HF band).[4]

Previous reports on HRV or power spectrum analysis have reported that the abnormalities in ALS include increased sympathetic tone or impaired cardiovagal function, both of which lead to the sympathovagal imbalance. Pisano et al. first reported the imbalance of sympathovagal function due to an increase in LF/HF ratio.[6] This alteration was not related to the clinical features or disease duration. Similar observations were made by other researchers.[4,19–21] For example, Linden et al. observed reduced baroreflex sensitivity and diminished cardiorespiratory transfer during normal breathing in ALS due to decreased LF and HF bands, respectively.[4] These findings were similar to those reported for essential hypertension sharing a common central autonomic derangement. By using sinusoidal neck suction method to stimulate carotid baroreceptors, Hilz et al. showed impaired cardiovagal response with preserved sympathetic vasomotor control.[19] In addition, in early stage of ALS, patients with bulbar involvements show more predominant cardiovagal dysfunction than patients with non-bulbar involvement.[20]

Other Observations

A limited number of reports have shown decreased sympathetic outflow in patients with ALS. The corrected QT interval (QTc) on electrocardiography (ECG), a measure of sympathetic influence on the heart, was reported to be prolonged in length and increased in dispersion in patients with ALS, indicating reduced sympathetic activity.[22] It should be noted that this report did not evaluate the extent of sympathetic tone and the co-existence of constantly increased sympathetic tone and QT interval prolongation at the terminal stage of ALS does not seem contradictory. Another evidence for impaired cardiac sympathetic function was provided by a ^{123}I-metaiodobenzylguanidine (MIBG) uptake study by Druschky et al., which evaluated the function of the postganglionic sympathetic terminals in the heart. These authors studied MIBG-single photon emission computed tomography in early stage of ALS and found decreased heart/mediastinum ratio (mean 1.82 ± SD 0.27) in patients with ALS compared to normal controls (2.16 ± 0.26).[23] They concluded the presence of cardiac postganglionic sympathetic denervation. However, there are no similar reports to date and their conclusion needs further validation.[24]

Figure 1: Diurnal Variations of Blood Pressure and Heart Rate in a Patient in an Advanced Stage on Ventilator



Daytime hypertension with tachycardia was followed by nocturnal pressure falls without counter-regulation of hear rate. Source: reproduced with permission from Shimizu et al., 1994.[10]

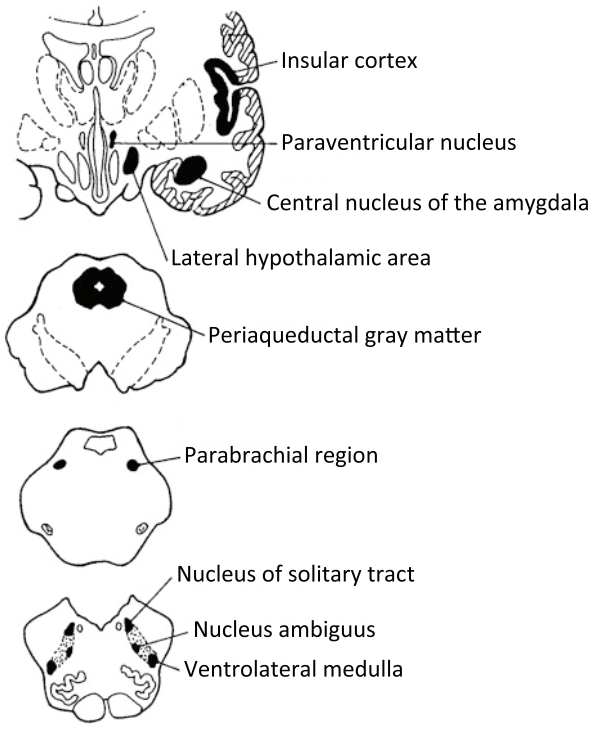
Observations in Advanced Stage of Amyotrophic Lateral Sclerosis

Although the abovementioned autonomic dysfunctions are usually subclinical, patients in most advanced stage of ALS, when ventilators are used, often show critical autonomic manifestations. Marked fluctuation of blood pressure and heart rate, namely 'autonomic storm', may occur during clinically stable stage on ventilator. The autonomic storm presents paroxysmal hypertensive crisis: more than 250 mmHg of systolic blood pressure and tachycardia without counter-regulation of heart rate (see *Figure 1*).[10,13,25] During the hypertensive crisis, plasma norepinephrine level is usually markedly high, indicating sympathetic hyperactivity. The presence of tachycardia strongly suggests central resetting of baroreflex sensitivity. Patients often exhibit facial flushing, jaw clonus-like involuntary movements, pseudobulbar affect, and disinhibition of emotional expression during the crisis. These observations may suggest a limbic origin for the autonomic storm, although the precise pathophysiology remains to be elucidated.

The hypertensive stage is often followed by successive blood pressure fall (circulatory collapse) without counter-regulation of heart rate, which may lead to sudden death.[10] The circulatory collapse is likely to occur during sleep at night and hyposensitivity of peripheral adrenoceptors might enhance the pressure decrease by a sleep-associated decrement of sympathetic tone.[13] The adrenoceptor hyposensitivity is probably attributed to its down-regulation due to the constant high-level secretion of norepinephrine from sympathetic nerve terminals.

All these features resemble symptoms of baroreflex failure, whose cardinal manifestations include hypertensive crisis, volatile or labile hypertension, orthostatic tachycardia and malignant vagotonia.[3] Similar symptoms are also found in the acute stage of Guillain-Barré syndrome, which involves vagal afferent and sympathetic preganglionic efferent nerves.[26,27] Differential diagnosis of baroreflex failure includes

Figure 2: Schematic Illustrations of the Central Autonomic Network Inducible of Sympathetic Hyperactivity



Source: reproduced with permission from Benarroch, 1993.[46]

central nervous disorders, psychological or metabolic diseases such as pheochromocytoma and disorders of peripheral baroreflex deafferentation. A recent study on patients having ALS with circulatory collapse showed a quantitative preservation of vagal visceral branches, excluding the possibility of peripheral deafferentation of baroreflex arc.[28] Although there have been reports of pathological involvement of peripheral sensory and small fibers in ALS,[29,30] the autonomic storm cannot be attributed to such peripheral nervous lesions.

Disease Specificity of Autonomic Storm

The question to be resolved is whether the autonomic storm is primary or secondary to ALS. The factors that should be considered are the long-term bedridden state, severe muscle atrophy, long-term ventilator support and psychological stress.[10] At present, there is no evidence that certainly supports or rules out these potential effects. However, autonomic storm in ALS is a very peculiar phenomenon that seldom occurs in other neuromuscular disorders. Given that not all patients who have ALS with a long-term use of ventilator and severe muscle wasting develop autonomic storm and that ventilator-dependent patients with Duchenne muscular dystrophy having similar muscle atrophy do not usually show any circulatory fluctuation, the autonomic storm may be primary to ALS.[10]

Sudomotor Function

Patients with ALS often complain of increased or reduced sweating in their hands or feet, altered skin temperature, or skin discolouration. Studies of sudomotor function have reported that denervation of sympathetic postganglionic cholinergic nerves could occur in ALS. The sudomotor axon reflex test showed reduced sweating rate in ALS, indicating mild postganglionic sudomotor dysfunction.[31,32] On the other hand, Beck et al. documented that compared to healthy subjects, patients in early stage of ALS had higher sweating rate in the palms, potentially supporting the idea of increased central sympathetic tone in ALS.[33] However, they also reported a reduced sweating rate in advanced stage of ALS, suggesting progressive peripheral sudomotor dysfunction and resultant atrophy of sweat glands.

In addition, studies have electrophysiologically evaluated the sweat function by using sympathetic skin response (SSR) and skin sympathetic nerve activity (SSNA).[34–38] SSR was reported to be abolished in 40 % of the patients in early stage of ALS and its latency was prolonged, suggesting subclinical involvement of sudomotor fibres in ALS.[35] Interestingly, in contrast to these reports of SSR, SSNA assessment by microneurography showed high resting frequency and reduced response to mental stress, accompanied by slight prolongation of SSNA reflex latencies.[34,38] These findings support basic sympathetic hyperactivity of central origin. The reduced response may reflect the 'ceiling effect' as in the case of MSNA.[14] Both the vasomotor and sudomotor systems might be hyperactive, even though some patients show peripheral sudomotor hypofunction.

Neuropathology

Neuropathological studies on the autonomic dysfunction in ALS have been limited. IML of the thoracic spinal cord, one of the most efferent neurons of the baroreflex arc, was reported to be moderately decreased in number in patients with ALS.[39,40] This reduction has been often considered as a potential cause of the sympathetic dysfunction, although the reduction of IML neurons itself does not explain the increase in sympathetic tone. It may explain the reduced size or delayed latency of SSR in ALS and it is not contradictory to the idea of sympathetic hyperactivity shown in ALS. The reported reduction of IML neurons in ALS is not severe and the remaining neurons are considered hyperactive. Consistent with this, IML, dorsal vagus nucleus and solitary tract nucleus are well preserved in most advanced stage of ALS,[41,43] when a considerable number of patients who are on ventilator develop ophthalmoplegia or a totally locked-in state.[41,42]

There are a few reports on the neuropathology for sympathetic hyperactivity, especially in the brainstem and the limbic system. Shimizu et al. and Kato et al. reported that some patients with blood pressure fluctuation in the advanced stage of ALS showed lesions in the lateral hypothalamus, the central and basolateral nuclei of the amygdala and the brainstem reticular formation.[10,43] It should be noted that these pathological findings were variable and infrequent across patients. The primary autonomic centres in the medulla oblongata, however, were intact. Although the brainstem reticular formation was previously suggested to be primarily involved in ALS,[44] Kato et al. observed preservation of catecholaminergic neurons in the medullary reticular formation in patients with ALS and blood pressure fluctuation.[45]

It will be difficult to neuropathologically elucidate the precise central lesions corresponding to the clinical autonomic manifestations. Generally, the central autonomic neural control is very complex and subtle imbalance between excitatory and inhibitory pathways could activate various autonomic nuclei and induce autonomic symptoms.[46,47] If destruction of nervous tissue is severe, the lesions could produce constant autonomic symptoms like orthostatic hypotension in multiple system atrophy, but the fluctuations of symptoms may be attributed to mild alterations that cannot be detected by classic neuropathological methodology. Recent advancement in TDP-43 proteinopathy for

sporadic ALS revealed a wide distribution of the protein aggregates in the central nervous system,[48] including the limbic system, also called the 'central autonomic network' (see *Figure 2*).[46] In addition, the limbic motor and bulbo-respiratory motor systems are anatomically and physiologically linked to each other.[49] Furthermore, ALS is often accompanied by involvement of the frontotemporal lobes, which overlaps the limbic system. However, there have been no reports on a correlation between cognitive dysfunction and sympathetic tone, and further studies are needed.

Prognostic Significance

For patients in early stage of ALS, sympathetic hyperactivity rarely influences survival. A major cause of death is usually respiratory muscle paralysis, not circulatory collapse. Pinto et al., however, reported that decreased variability of heart rate might predict sudden death (cardiac arrest) in ALS.[50] Four patients in their report showed very low values of the heart rate coefficient variation (<0.20), and three of them died suddenly within the following two months, despite normal nocturnal oxygenation. The prolongation of QT interval on ECG may also be an indicator of poor prognosis.[22] The autonomic storm or circulatory collapse in advanced stage of ALS with ventilator use predicts poor survival prognosis. Patients with hypertensive crisis may die within several months, if not treated appropriately.[10] Ventilator-dependent patients showing blood pressure fluctuation should be continuously monitored for vital signs, especially at night, because sudden pressure fall is more likely to occur during sleep.

Familial Amytrophic Lateral Sclerosis

Recent development of gene analysis in familial ALS has disclosed many types of gene abnormalities. Although precise distributions of disease-specific central nervous lesions have not been elucidated in each type of familial ALS, superoxide dismutase 1 (SOD1)-related ALS may show variable symptoms and lesions across families or locations of mutation. Some patients with SOD1-related ALS show severe degenerative lesions in the autonomic nuclei, including IML, dorsal vagus nucleus and solitary tract nucleus. Autonomic failure, such as orthostatic or postprandial hypotension and atonic bladder, was reported to develop in a sporadic case of SOD1-related ALS (V118L).[51] Since cases of classical sporadic ALS never showed autonomic failure, sporadic ALS and SOD1-related ALS must have pathogenic and phenotypic differences. Like classic sporadic ALS, SOD1-related ALS may also show sympathetic hyperactivity. A ventilator-using patient with G93S mutation showed paroxysmal hypertension, prominent sensory disturbances and pressure fall during sleep at night.[52] The norepinephrine level was very high and the bladder function showed an overactive detrusor type. In addition, Kandinov et al. reported a higher heart rate at rest and following stress in transgenic mice carrying a SOD1-G93A mutation than in wild-type mice.[53] Blood pressure elevated even before the appearance of motor dysfunction and gradually decreased with the progress of the disease.[54] Thus, SOD1-related ALS may show variable phenotypes of autonomic dysfunction across different genotypes.

Treatments

Therapeutic approaches for the sympathetic hyperactivity in ALS have not yet been established. Since most patients with ALS die from respiratory failure, it has not been clarified whether treatment of the sympathetic hyperactivity would bring a better effect on disease progression or survival. If the sympathetic tone is one of the prognostic indicators, it should be treated by medicine such as beta-adrenergic antagonists. No evidence, however, has been obtained so far. Avoiding hypoxic episodes may reduce reflex sympathetic activation.[55] Hypertensive crisis or circulatory collapse in advanced stage of ALS should be immediately treated, given the severe and poor prognosis it indicates. Direct vasodilators, such as calcium antagonists, however, should be avoided since these may induce critical over-reduction of the blood pressure. Ohno et al. reported that tamsulosin hydrochloride, by acting as central alpha-adrenergic blocker, might modulate blood pressure and reduce plasma norepinephrine level.[9] Benzodiazepines might also prove effective for centrally increased sympathetic tones by potentially mediating central GABAergic functions.[3,25] To establish an appropriate therapy, further studies are needed.

Conclusion

The cardinal features of the autonomic nervous dysfunction in ALS are the sympathetic hyperactivity and sympathovagal imbalance. Their clinical significance is obscured in early stage, but critical in advanced stage of the disease, when ventilators are required. Details of the involved central nervous lesions and appropriate therapeutic approaches remain to be determined. No evidence has been found to date of the pathophysiological correlation between the neurodegenerative process of ALS and the autonomic abnormalities. Further studies may be needed to establish the pathognomonic significance of autonomic dysfunction in ALS. ■

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NEUROPATHOLOGY

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Case Report

A Japanese patient with familial ALS and a p.K510M mutation in the gene for FUS (*FUS*) resulting in the totally locked-in state

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We describe a Japanese patient with familial amyotrophic lateral sclerosis (ALS) and a p.K510M mutation in the fused in sarcoma gene *(FUS)*. The patient's condition was characterized clinically by an early onset and rapid progression. The patient eventually required mechanical ventilation and progressed to the totally locked-in state. Neuropathologically, multiple system degeneration with many FUS-immunoreactive structures was observed. The involvement of the globus pallidus, subthalamic nucleus, substantia nigra, cerebellar efferent system, and both upper and lower motor neurons in the present patient was comparable to that described for ALS patients with different mutations in *FUS*, all of whom progressed to the totally locked-in state. However, the patient also exhibited degeneration of the cerebellar afferent system and posterior column. Furthermore, the appearance of non-compact FUS-immunoreactive neuronal cytoplasmic inclusions and many FUS-immunoreactive glial cytoplasmic inclusions were unique to the present patient. These features suggest that the morphological characteristics of the FUS-immunoreactive structures and distribution of the lesions vary with the diversity of mutations in *FUS*.

Key words: basophilic neuronal cytoplasmic inclusions, familial amyotrophic lateral sclerosis, fused in sarcoma, multiple systems involvement, totally locked-in state.

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INTRODUCTION

There have been accumulating reports of autopsied cases of amyotrophic lateral sclerosis (ALS) in which neuronal cytoplasmic inclusions (NCIs) and/or glial cytoplasmic inclusions (GCIs) that are immunoreactive for fused in sarcoma (FUS) appear in extensive areas beyond the motor neuron system.[1–8] It is noteworthy that some of these cases showed neuron and/or fiber loss in multiple systems.[1,3,6,7] In our previous report of two Japanese sisters with a p.P525L mutation in the gene for FUS (*FUS*),[6] the elder sister progressed to a totally locked-in state[9] (communication ability stage V[10]), required mechanical ventilation, and showed severe degeneration in multiple systems, while the younger sister died in the early stages of the disease. The findings from neuropathological examinations revealed that the neuronal loss was preceded by the appearance of FUS-immunoreactive NCIs and that the characteristic feature in the elder sister was severe degeneration of the frontal lobe and striatum.

Here, we describe another patient with familial ALS who progressed to the totally locked-in state. The patient had a p.K510M mutation in *FUS* and exhibited degeneration of multiple systems. This is the first report of a detailed clinicopathological examination of an ALS patient with a p.K510M mutation in *FUS*, although ALS in a patient with a p.K510E mutation in *FUS* was reported clinically as early onset with involvement of the proximal muscles, a predominance of lower motor neuron signs, and rapid progression.[11] We discuss the differences between previously described patients with mutations in *FUS* who progressed to

the totally locked-in state and showed multiple system degeneration.

CASE REPORT

Clinical summary

A 39-year-old Japanese man (Subject II-5 in Fig. 1) developed weakness in his left leg and then noticed fasciculation of his right leg. He developed quadriparesis, neck weakness, dysphagia and dysarthria. One year and 8 months after the onset of the disease, he underwent a tracheostomy with mechanical ventilation and a percutaneous endoscopic gastrostomy. Neurologically, the patient was alert and the deep tendon reflexes were absent from all extremities. At 6.5 years after appearance of his first symptoms, he became quadriplegic with bilateral abduction paresis of the extraocular muscles, and he had difficulty in urinating. Finally, voluntary movement disappeared totally and he was unable to communicate by any means (communication ability stage V[10]). He died of pneumonia at the age of 47, 8.5 years after onset.

The elder brother of the present patient (Subject II-1 in Fig. 1) died of ALS, which had a similarly rapid progressive clinical course, in his third decade.

Genetic analysis

DNA was extracted from the patient's leukocytes by using a conventional method after the patient provided informed consent. All of the exons and exon–intron boundaries of the gene for FUS, copper/zinc superoxide dismutase *(SOD1)*, transactivation response DNA-binding protein of 43 kDa *(TDP-43)*, and optineurin (*OPTN*) were examined by direct sequencing of PCR products.[6]

Genetic analysis identified a heterozygous missense mutation of c.1529A>T at codon 510 (p.K510M) in *FUS* but did not identify mutations in the exons or at the exon–intron boundaries of the other genes.

METHODS

The specimens from the brain and spinal cord were fixed with formalin and embedded in paraffin. Neuronal loss and/or fiber loss and gliosis were assessed in various regions of the nervous system by using 10-μm-thick sections and HE and KB stains. When necessary, Bodian, Gallyas–Braak or Holzer staining was performed.

For immunohistochemistry, 6-μm-thick sections were prepared. Sections from the frontal lobe, medial temporal lobe of the hippocampus, and pons were immunostained for phosphorylated TDP-43 (p-TDP-43), using a rabbit polyclonal antibody against phosphoTDP-43 (Cat. No. TIP-PTD-P01 pS409/410-1; CosmoBio, Tokyo, Japan) at a dilution of 1:3000. Sections from the cerebral lobe, basal ganglia, cerebellum, brainstem, and spinal cord were immunostained for FUS, using a rabbit polyclonal antibody against FUS (Cat. No. HPA008784; Sigma-Aldrich Japan, Tokyo, Japan) at a dilution of 1: 100. Prior to antibody incubation, sections were treated with microwaving in citrate-buffered saline (pH 9.0, 95°C, 500 W, 15 min) for antigen unmasking. Antibody binding was visualized by the labeled streptavidin-biotin immunoperoxidase method. The chromogen and counterstain were diaminobenzidine and hematoxylin, respectively. Immunoreaction product deposits were undetectable on sections from which the primary antibodies were omitted.

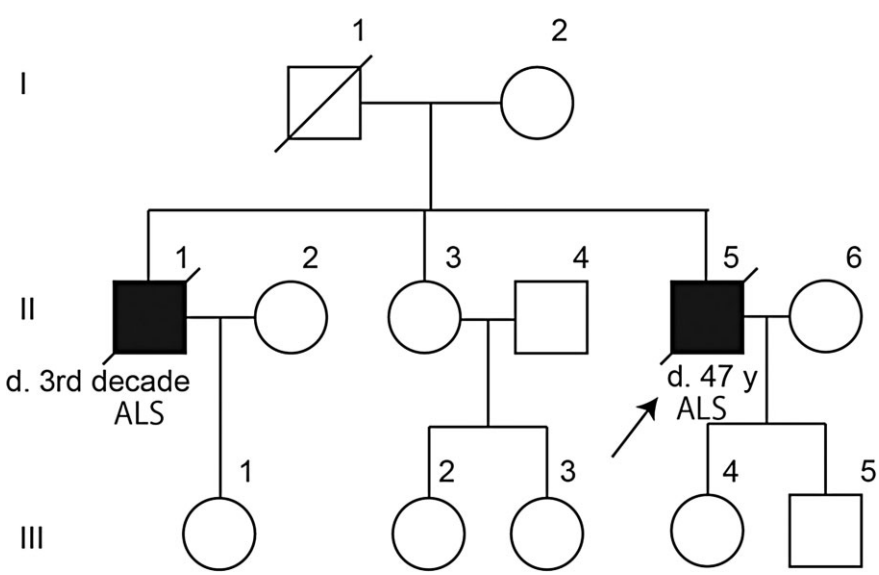

Fig. 1 Pedigree of the family. The arrow indicates the proband. The affected individuals are represented by the solid black square.

Pathological findings

The brain weighed 1233 g. While the frontal lobe and cerebellum displayed mild atrophy, the brain stem including the pontine base and the spinal cord showed marked atrophy. The brain stem motor nuclei and the anterior horn of the spinal cord showed marked neuronal loss with fibrillary gliosis. Bunina bodies, basophilic NCIs and p-TDP-43-immunoreactive inclusions were undetectable in the lower motor neurons. Betz cells were markedly depleted, accompanying a few small groups of microglia in the precentral cortex. The most remarkable finding of the examination of the present patient was the involvement of multiple systems as follows (Table 1): (i) marked degeneration and atrophy of the anterior and lateral funiculi of the spinal cord (Fig. 2A) and the brain stem tegmentum (Fig. 2B–D) including the reticular formation, superior colliculi, superior cerebellar peduncles and medial lemniscus; (ii) neuronal loss with gliosis in the globus pallidus, subthalamic nucleus (Fig. 2E), and substantia nigra; (iii) neuronal loss in the Clarke's nucleus, accessory cuneate nucleus, and dorsal root ganglia, and fiber loss in the middle root zone of the posterior column and spinocerebellar tract (Fig. 2A); (iv) neuronal loss with gliosis in the cerebellar dentate nucleus, red nucleus (Fig. 2C), and inferior olivary nucleus (Fig. 2D), and fiber loss with many microglia in the superior cerebellar peduncles; and (v) neuronal loss in the pontine nuclei and fiber loss with many microglia in middle cerebellar peduncles (Fig. 2C) and cerebellar white matter. One to three basophilic NCIs, identified by HE staining (Fig. 2F), were observed in the pontine nuclei, caudate nuclei, cerebellar dentate nucleus, globus pallidus, and frontal cortex on each section. The basophilic NCIs were stained with Bodian staining. Some of the basophilic NCIs and GCIs were detected with Gallyas-Braak staining. The basophilic NCIs

Table 1 Neuropathological findings

	Degeneration	FUS immunoreactivities	
		NCIs	GCIs
Motor neurons			
Primary motor cortex	+	++	++
Corticospinal tract	++	n	++
Anterior horn of the spinal cord	+++	−	+
Hypoglossal nucleus	+++	−	−
Oculomotor nucleus	+++	−	−
Spinal cord			
Clarke's nucleus	+++	+	−
Spinocerebellar tract	+++	n	−
Middle root zone of the posterior column	++	n	−
Intermediolateral horn	−	−	−
Anterior and lateral funiculi†	+++	n	−
Basal ganglia and substantia nigra			
Caudate nucleus	−	+	+
Putamen	−	+	++
Globus pallidus	++	+	+++
Subthalamic nucleus	+++	n	+++
Substantia nigra	+++	+	+++
Cerebellum and related areas			
Purkinje cells	+	−	−
Cerebellar dentate nucleus	++	++	+
Superior cerebellar peduncle	++	n	++
Red nucleus	++	++	++
Central tegmental tract	+	n	++
Inferior olivary nucleus	+	++	++
Pontine nucleus	++	++	++
Middle cerebellar peduncle	++	n	++
Cerebellar white matter	++	n	++
Cerebral cortex			
Frontal cortex	−	+	++
Frontal white matter	−	n	++
Insular cortex	−	+	+
Temporal cortex	−	+	+
Primary sensory cortex	+	+	++

Degeneration: neuron and/or fiber loss assessed on HE-, KB-stained sections. The degeneration is indicated as absent (−), slight (+), mild (++), or severe (+++). The FUS immunoreactivities are indicated as none (−), rare (+), occasional (++), or frequent (+++). † The region except the corticospinal tract. FUS, fused in sarcoma; GCIs, glial cyoplasmic inclusions; n, not evaluated; NCIs, neuronal cytoplasmic inclusions.

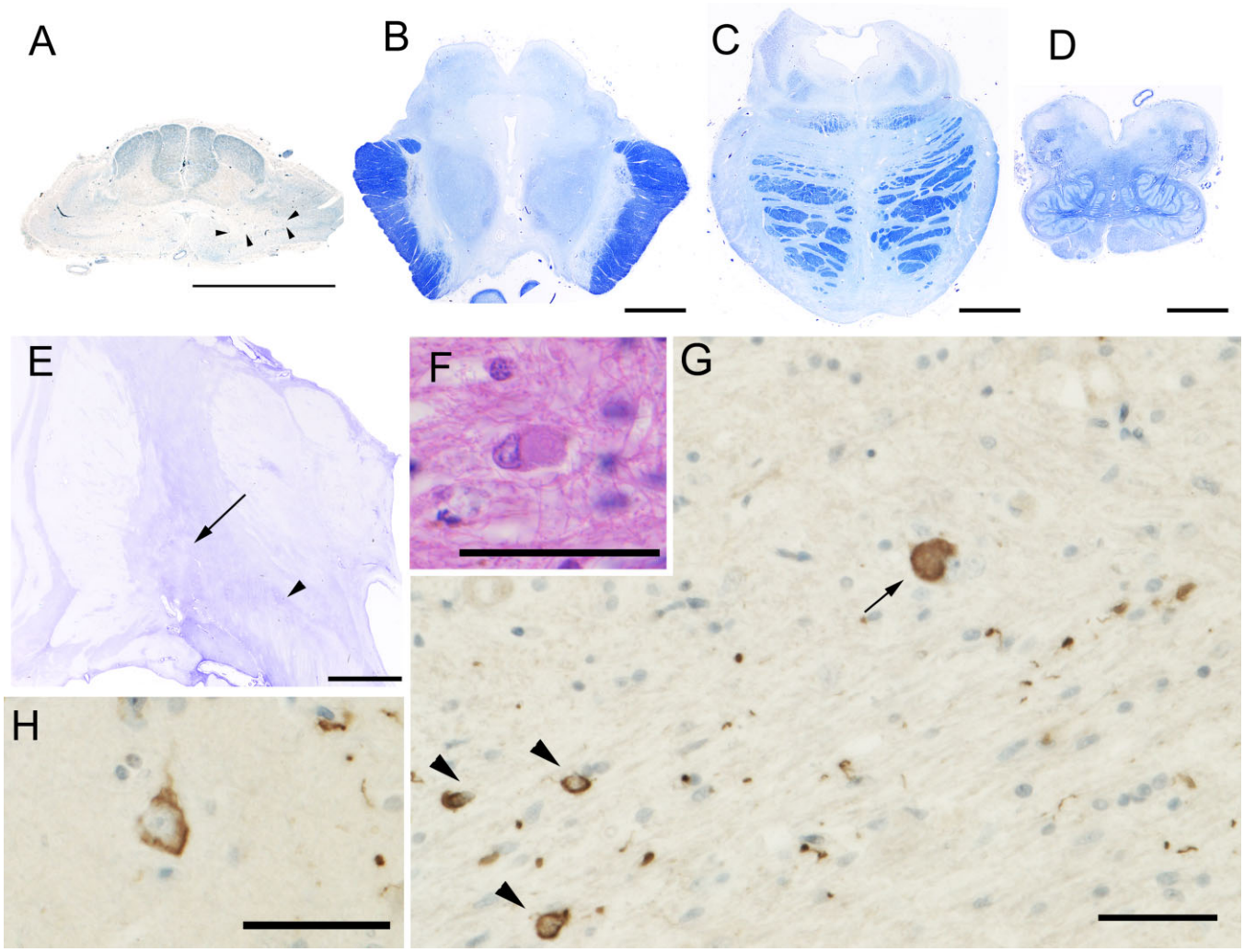

Fig. 2 Neuropathological findings. (A) The fifth cervical spinal cord showed marked loss of nerve fibers in the corticospinal tract, spinocerebellar tract, anterolateral funiculus, and middle root zone of the posterior column. The anterior horn showed marked atrophy (arrowheads). (B) Marked degeneration was observed in the tegmentum of the midbrain. (C) Marked nerve fiber loss in the superior and middle cerebellar peduncles and transverse fibers, marked degeneration of the tegmentum, and mild atrophy of the corticospinal and corticopontine tracts in the pons were observed. (D) The medulla oblongata showed marked degeneration. (E) The internal capsule, globus pallidus (arrow), and subthalamic nucleus (arrowhead) showed fibrillary gliosis. (F) Basophilic neuronal cytoplasmic inclusion (NCI) in the pontine nuclei. (G) The arrow indicates a fused in sarcoma (FUS)-immunoreactive NCI with a homogeneous morphology. Glial cytoplasmic inclusions (arrowheads) were observed in the pontine base. (H) Filamentous, FUS-immunoreactive NCI in the motor cortex. (A–D) KB staining; (E) Holzer staining; (F) HE staining; (G, H) Immunostained for FUS. Scale bar = 0.5 cm (A–E) and 50 μm (F–H).

were immunoreactive for FUS. FUS-immunoreactive NCIs and GCIs were numerous and widespread throughout the brain (Table 1). Most of the FUS-immunoreactive NCIs were the non-compact type and consisted of homogeneous (Fig. 2G), granular, or clustered thick filamentous materials (Fig. 2H). No FUS-immunoreactive intranuclear inclusions were observed.

DISCUSSION

The disease progression in the present patient, who had a p.K510M mutation in *FUS*, was relatively early-onset with predominantly lower motor neuron signs. This type of progression is similar to that of ALS patients with different mutations in *FUS*.[1,6,8,11–15] It is notable that the rapid progression, which led to the patient requiring mechanical ventilation and was followed by totally locked-in state, of the patient's clinical course was similar to the progression of disease in two previously described ALS patients with different mutations in *FUS* (Table 2).[1,6] This feature is consistent with the finding of our previous report, that patients with ALS who reach communication stage V require mechanical ventilation significantly earlier than patients who remain able to communicate.[10]

Table 2 Clinicopathological summary of patients with ALS who progressed to a totally locked-in state and had a mutation in the *FUS*

Patient [reference number]	Present patient	[1]	[6] Patient 1
Mutation in *FUS*	p.K510M	p.R521C	p.P525L
Family history	Yes	Yes	Yes
Gender	M	nd	F
Onset age (years)	39	31	13
Start of respiratory assistance (months)	20	9	18
Duration from onset to the totally locked-in state (months)	102	48	120
Disease duration (months)	102	168	312
Initial symptom	Lower extremities	Upper extremities	Lower extremities
Brain weight (g)	1233	1070	715
Neuropathological features (neuron and/or fiber loss)			
Motor neurons			
Primary motor cortex	+	+	++
Corticospinal tract	+++	+++	+++
Anterior horn of the spinal cord	+++	+++	+++
Frontal cortex	–	+++	++
Basal ganglia and substantia nigra			
Striatum	–	+	+++
Globus pallidus	+++	+++	++
Subthalamic nucleus	+++	+++	+++
Substantia nigra	+++	+++	+++
Cerebellum and related areas			
Cerebellar dentate nucleus (cerebellar efferent system)	++	+++	+++
Pontine nuclel (cerebellar afferent system)	++	+++	++
Middle cerebellar peduncle (cerebellar afferent system)	++	+	+
Limbic area			
Subiculum	–	nd	–
Entorhinal cortex	–	–	–
Posterior column			
Clarke's nucleus	++	+++	–
Middle root zone of the posterior column	++	++	–
Basophilic neuronal cytoplasmic inclusions (NCIs)	Rare	Many	Many
Morphology of the FUS-immunoreactive NCIs	Non-compact	Round	Small compact
FUS-immunoreactive glial cytoplasmic inclusions	Many	Many	Rare

ALS, amyotrophic lateral sclerosis; FUS, fused in sarcoma; nd, not described.

As shown in Table 2, the primary neuropathological features of the three patients was multiple system degeneration with many FUS-immunoreactive structures.[1,6] All of the patients showed involvement of the globus pallidus, subthalamic nucleus, substantia nigra, and cerebellar efferent system, in addition to severe degeneration of both upper and lower motor neurons (Table 2).[1,6] The degeneration of the posterior column that was observed in the present patient was reported as the characteristic feature of an ALS patient with a p.R521C mutation in *FUS* (Table 2).[1,3,7] Degeneration of the cerebellar afferent system that involved not only the pontine nuclei but also the middle cerebellar peduncles and cerebellar white matter was seen only in the present patient. The emergence of microglia in the cerebellar afferent system suggests that the degeneration started later here than in the other lesions. On the other hand, the degeneration of the frontal lobe and the striatum was only observed in the patient with a p.P525L mutation in *FUS*.[6]

In the present patient, HE staining identified only a few basophilic NCIs, known to be a hallmark of ALS with FUS pathology, whereas many FUS-immunoreactive NCIs were observed. In ALS patients with mutations in *FUS*, who had many basophilic NCIs, the FUS-immunoreactive NCIs were small and compact in patients with a p.P525L mutation[4,6] and round in patients with a p.R521C mutation (Table 2).[1,3,7] In contrast to the basophilic NCIs, the non-compact, FUS-immunoreactive NCIs observed in the patient were difficult to identify by HE staining. Recently, we examined an ALS patient with a p.R521G mutation in *FUS*. The patient had only a few subtle basophilic NCIs but many non-compact FUS-immunoreactive NCIs.[8] These findings suggest that not all of the ALS patients with FUS pathology have abundant basophilic NCIs. Furthermore, both the present patient and the reported patient with a p.R521C mutation in *FUS*[1] had many FUS-immunoreactive GCIs, while the patient with a p.P525L mutation[6] had only a few of these.

It is possible that while multiple system degeneration is characteristic of patients who progress to the totally locked-in state,[9,10] the morphological characteristics of the FUS-immunoreactive NCIs and the occurrence of FUS-immunoreactive GCIs may vary depending on the muta-

tion site in *FUS*. It is well known that the FUS gene is located on chromosome 16p11.2, and that the gene product FUS is a 53 kDa (526 amino acids) protein. The mutations of the patients discussed in this report were all located within the proline/tyrosine-nuclear localization signal (PY-NLS) domain of FUS. Patients with mutation near the C-terminal of the PY-NLS (p.P525L) seemed to be younger at the time of onset, progress to respiratory failure more rapidly, and have more compact NCIs and fewer GCIs than patients with mutation in the opposite side (p.R521C, p.K510M).

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REPORT

An autopsy case of familial amyotrophic lateral sclerosis with *FUS* R521G mutation

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Introduction

To date, several amyotrophic lateral sclerosis (ALS) patients with the *fused in sarcoma (FUS)* gene mutation have been reported. Detailed clinicopathological investigations of patients with the *FUS* R521C mutation have revealed the following clinical characteristics: weakness of the neck and proximal muscles (1–4) and neuropathological findings with multiple system involvement, in addition to motor neuron degeneration (1,4–6). Although the *FUS* R521G mutation has been detected in familial ALS patients (7,8), it is unknown whether their clinicopathological characteristics are the same as those patients with the *FUS* R521C mutation. Here, we report the first detailed clinicopathological findings of a familial ALS patient with the *FUS* R521G mutation.

Case report

A 67-year-old Japanese female was aware of breathlessness. Eight months later, she developed weakness of the upper extremities and body weight loss, followed by weakness of the neck. She was admitted to our hospital because of dysphagia and progression of her symptoms 14 months after onset. Neurological examination revealed bulbar palsy, weakness of neck muscles, proximal muscle weakness of upper extremities, patellar hyperreflexia, and respiratory insufficiency. No movement disorders, including rigidity, tremor and akinesia were seen. She died of respiratory failure after 15 months of illness. DNA was extracted from the patient's leukocytes with informed consent. Sequence analysis of the *FUS* gene (9) identified an arginine 521 to glycine (R521G) mutation. Her father had died of pneumonia with dysarthria at the age of 36 years, and her brother died of ALS at the age of 33 years after one year's illness.

Post mortem examination showed a mildly swollen brain (weight 1395 g). Spinal cord and ventral roots were slightly atrophic. Microscopically, the anterior horn (Figure 1A, B) and the hypoglossal nucleus showed moderate neuronal loss (Table I). In the precentral cortex, Betz cells were slightly decreased and small groups of lipofuscin-laden macrophages (Figure 1C) were scattered. Large-sized myelinated fibers were slightly decreased in the corticospinal tract. Mild neuronal loss was recognized in the substantia nigra (Figure 1D). Clarke's nucleus and posterior horn of the spinal cord showed mild gliosis (Table I). Neither Bunina bodies nor TAR DNA-binding protein 43-immunoreactive structures were identified. Few subtle basophilic neuronal cytoplasmic inclusions (NCIs) were observed in the anterior horn of the spinal cord (Figure 1B) and the subthalamic nucleus. There were ubiquitin-immunoreactive granular cytoplasmic inclusions (Figure 1E) in the anterior horn of the spinal cord, while the round NCIs had no ubiq-

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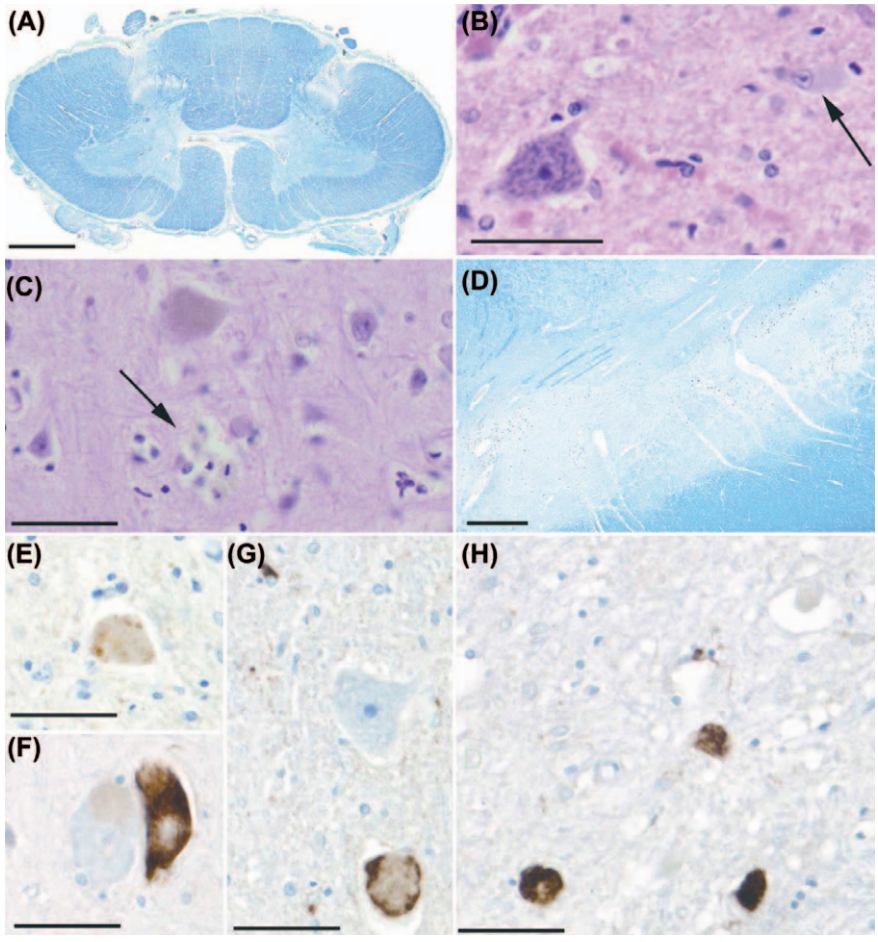

Figure 1. Histological evaluation of clinicopathological features. A: Mild atrophy was observed at the eighth cervical cord (C8). The anterior horn was mildly atrophic (Klüver-Barrera (KB) staining). B: Moderate neuronal loss and gliosis and subtle basophilic neuronal cytoplasmic inclusion (NCI) (arrow) were observed in the anterior horn at C8 (haematoxylin and eosin (HE) staining). C: Betz cells were mildly decreased and small groups of lipofuscin-laden macrophages (arrow) were scattered (HE staining). D: Mild neuronal loss was observed in the substantia nigra SN (KB staining). E: Ubiquitin-immunoreactive granular neuronal cytoplasmic inclusion (NCI) was observed (ubiquitin: DAKO, 1:600). F: Fused in sarcoma (FUS)-immunoreactive Betz cell was observed (FUS: Sigma, 1:100). G: FUS-immunoreactive NCI and FUS-immunoreactive glial cytoplasmic inclusion were observed. H: Several FUS-immunoreactive NCIs were seen in the subthalamic nucleus. (E, G: anterior horn at C8; Bar in A and B = 1 mm, Bar in B, C, E–H = 50 μm).

uitin immunoreactivity. FUS-immunoreactive NCIs were found in Betz cells (Figure 1F) in addition to lower motor neurons (Figure 1G) and non-motor neurons (Figure 1H). Their morphological characteristics included cytoplasmic staining (4) and granular or clustered thick filaments in the cytoplasm (Figure 1F–H). No FUS-immunoreactive intranuclear inclusions were observed.

Discussion

The feature of rapid progression of proximal-dominant muscle weakness in our patient was similar to that of patients with the *FUS* R521C mutation (Table II) (1–6). Neuropathologically, the present patient showed mild neuronal loss in the substantia nigra in addition to severe lower motor neuron degeneration and mild upper motor neuron degeneration. Furthermore, FUS-immunoreactive NCIs and glial cytoplasmic inclusions were observed not only in these areas, but also in other areas with no neuronal loss (Table I). Autopsy reports on affected family members of varying disease duration with the *FUS* mutation showed that FUS-immunoreactive structures have an important role in degeneration (4,9). In the present patient, although the disease duration was only 15 months without ventilatory support, degeneration of multiple systems had already occurred beyond motor neurons. These results suggested that degeneration started from the substantia nigra, which showed neuronal loss, and extended to the subthalamic nucleus, which had many FUS-immunoreactive structures. Furthermore, areas with occasional FUS-immunoreactive structures such as Clarke's nucleus, the middle root zone of the posterior column of the spinal cord, and the globus pallidus might be involved. These lesions were found in patients with the FUS R521C mutation (Table II). However, the FUS-immunoreactive NCIs in the present patient were morphologically different from cytoplasmic round inclusions found in other patients with the

Table I. Distribution of lesions and fused in sarcoma (FUS) immunoreactivities.

	Neuron or fibre loss/gliosis	*FUS* immunoreactivities	
		NCIs	GCIs
Motor neurons			
Primary motor cortex	+/−	+	−
Corticospinal tract	+/+	n	+
Anterior horn of the spinal cord	++/++	++	++
Hypoglossal nucleus	++/++	+	+
Oculomotor nucleus	−/−	−	−
Spinal cord			
Clarke's nucleus	−/+	+	+
Middle root zone of the posterior column	−/−	n	+
Spinocerebellar tract	−/−	n	−
Intermediolateral horn	−/−	+	−
Anterior and lateral funiculi*	+/+	n	+
Posterior horn	−/+	+	+
Basal ganglia and substantia nigra			
Caudate nucleus	−/−	−	+
Putamen	−/−	−	+
Globus pallidus	−/−	+	+
Subthalamic nucleus	−/−	++	+
Substantia nigra	+/+	+	+

The cerebral cortex except precentral cortex, hippocampus, thalamus, cerebellum and related areas were preserved with no FUS-immunoreactive structures.
Neuronal or fibre loss and gliosis were indicated as absent (−), mild (+), or moderate (++).
FUS pathology was indicated as none (−), occasional (+), or frequent (++), n: unable to evaluate. NCIs: neuronal cytoplasmic inclusions, GCIs: glial cytoplasmic inclusions.
*Regions except the corticospinal tract.

Table II. Clinicopathological features of patients with *fused in sarcoma (FUS)* R521G mutation and autopsy confirmed patients with *FUS* R521C mutation.

FUS mutation	R521G			R521C						
Patient (references)	Present patient	(7)	(8)	(1)			(4)		(5)	(6)
Onset (years)	67	ND	32	36	28	30	42	48	62	31
Disease duration (months)	15	ND	Alive	12	24	108	36	12	41	168
Proximally dominant weakness	+	ND	ND	+	ND	–	+	ND	+	+
Motor neuron degeneration*	LMN > UMN	LMN and UMN		LMN > UMN	LMN only		LMN and UMN		LMN > UMN	LMN and UMN
Degeneration* besides motor neuron system	SN, Clark, posterior horn	ND		Cuneate tract	SN, GP, Put, Cau	SN, MRZ, Purkinje cell	SN, GP Clark, MRZ	SN, STN, GP, Clark, MRZ, Cerebel	ND	SN, STN, GP, Clark, MRZ. Cerebel, CerbC
Basophilic NCIs	Equivocal	ND		ND	ND	+	+	+	+	+
FUS immunoreactivities				ND	ND					
NCIs	Cytoplasmic staining	Cytoplasmic staining					Earlier: Cytoplasmic staining, Later: Cytoplasmic inclusion		Cytoplasmic inclusion, or granular, collections of filaments	Cytoplasmic inclusion
GCIs	+	ND				+	+	+	+	+
FUS immunoreactivities areas without neuron or fibre loss	STN, GP, Put, MRZ	ND		ND	ND	LC	Thal, LC, Cerebel	Put, Cau	Spinal cord**	Hippocampus

LMN: lower motor neuron, UMN: upper motor neuron; *Degeneration: neuron and/or fibre loss and/or gliosis; NCIs: neuronal cytoplasmic inclusions; GCIs: glial cytoplasmic inclusions; +SN: substantia nigra; Clark: Clarke's nucleus; STN: subthalamic nucleus; GP: globus pallidus; Put: putamen; MRZ: middle root zone of the spinal posterior column; ND: not described; Cau: caudate; Thal: thalamus; LC: locus ceruleus; Cerebel: cerebellum and related areas; CerebC: cerebral cortex; **Spinal cord: dorsal grey matter and white matter of the spinal cord.

FUS R521C mutation (3,4–6), but were similar to the early stage NCIs (4).

In this study, we describe several clinical and pathological similarities between patients with the *FUS* R521G or *FUS* R521C mutation. These findings suggest that replacement of amino acid 521 arginine (R) to cysteine (C) or glycine (G) does not play a definite role in phenotypic difference in the pathologies seen with the above *FUS* mutations.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Marked preservation of the visual and olfactory pathways in ALS patients in a totally locked-in state

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Key words
amyotrophic lateral sclerosis – totally locked-in state – brain-machine interface – optic pathway – olfactory pathway

Abstract. Materials and methods: The present paper examines the brains and spinal cords in 7 patients with amyotrophic lateral sclerosis (ALS) receiving artificial respirator support in a totally locked-in state (TLS) neuropathologically in order to clarify whether any anatomical structures in the central nervous system are preserved. Results and conclusion: We found that the visual and olfactory pathways, hypothalamus, nucleus basalis of Meynert, and commissura anterior were remarkably well preserved, whereas the somatosensory, auditory, and gustatory pathways in the brain stem and/or spinal cord showed severe deterioration.

Introduction

Amyotrophic lateral sclerosis (ALS) is a disease mainly involving the upper and lower motor neurons in elderly humans. Many affected patients develop difficulty in communication due to weakness of the bulbar and skeletal muscles. For such patients, many types of communication devices, such as computers controlled by wrinkling the brow muscles or blinking, and communication boards, have been developed to assist augmentative and alternative communication (AAC). However, some ALS patients who require respirator support for many years fall into a totally locked-in state (TLS) as muscle weakness progresses. TLS is a state in which the patients cannot communicate by any means.

Recently, various brain-machine interfaces (BMIs) have been developed in an attempt to communicate with totally locked-in ALS patients by detecting alterations of intracerebral blood circulation or changes in electroencephalogram traces [1, 2, 3]. With regard to input of information to the brains of TLS patients, little is known about whether any sensation is preserved in the patients, and no previous study has investigated which anatomical pathways are preserved in the brain. The present study was conducted to clarify whether or not some neural pathways are preserved in the brains of patients with ALS who have fallen into a TLS.

Methods

The brains and spinal cords of the patients were fixed in 10% formalin, and multiple tissue blocks were embedded in paraffin. Histological examinations were performed on 6-μm-thick sections. These sections were stained with hematoxylin and eosin (H & E), Klüver-Barrera (K-B), or periodic acid-Schiff (PAS), and immunostained for synaptophysin (mouse monoclonal, clone 171B5, dilution 1 : 200, MBL, Nagoya, Japan), phosphory-

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Table 1. Examined cases.

Patient No.	Onset (yo)	Start TPPV (yo)	Duration of TPPV (yrs)	Duration of illness (yrs)	Death (yo)	Gender	Clinical diagnosis	Family history	Abnormal gene	Dementia	Initial symptom	Communicative ability stage*5	Cause of death	Brain weight (g)
1	13	14	26	27	40	F	Familial ALS	+	FUS*1	–	L	V	Pneumonia	715
2	52	53	13	14	66	F	ALS	–	n.e.	–	U	V	Pneumonia	783
3	57	59	9	11	68	M	Familial ALS	+	SOD1*2	–	B	V	Colon cancer	1,170
4	60	61	8	9	69	M	ALS	–	n.e.	–	U	V	Colon cancer	1,050
5	38	38	8	8	46	F	ALS	–	SOD1*3	–	L	V	Unknown (Sudden death)	1,019
6	39	41	6	8	47	M	Familial ALS	+	FUS*4	–	L	V	Pneumonia	1,233
7	64	65	3	5	69	M	ALS	–	n.e.	–	U	V	Pneumonia	1,190

yo = years old; yrs = years; F = female; M = male; ALS = amyotrophic lateral sclerosis; L = lower limb weakness; U = upper limb weakness; B = bulbar paresis; *1Fused in sarcoma P525L mutation, *2Cu/Zn superoxide dismutase (SOD1) Cys146Arg mutation; *3SOD1 Val118Leu mutation; *4Fused in sarcoma K510M mutation [8]; *5Ref. [6].

lated neurofilament (SMI-31: mouse monoclonal, dilution 1 : 3,000, Covance, Berkley, USA), cystatin C (rabbit polyclonal, dilution 1 : 1000, DAKO, Glostrup, Denmark), SOD1 (mouse monoclonal, dilution 1 : 5,000, MBL, Nagoya, Japan) or phosphorylated(p)TDP-43 (mouse monoclonal, clone TIP-PTD-M01, dilution 1 : 10,000, COSMO BIO, Tokyo, Japan).

Immunohistochemical staining was performed by the avidin-biotin-peroxidase complex (ABC) method (Vectastain ABC Elite kit, Vector, Burlingame, CA, USA). Non-specific binding of the biotin-avidin system reagents was blocked by pretreating the sections with 0.3% hydrogen peroxide in methanol and a normal blocking serum, and then incubating them with the required primary antibody overnight (for 17 hours) at 4 °C. The sections were then incubated for 1 hour with the secondary reagent containing biotinylated anti-rabbit or anti-mouse IgG (diluted 1 : 200) at 37 °C, and finally with the ABC solution for 1 hour at room temperature (RT). The sections were subjected to peroxidase reaction with 30 µL ImmPACT$^{T\text{-}M}$DABChromogen concentrate (Vector, Burlingame, CA, USA) (diluted 1 : 2, by 50 mM Tris HCl (pH 7.6)) in 1 mL ImmPACT^{TM} Diluent (Vector) for 5 minutes at RT.

Antigenicity was increased for SMI31 immunostaining by boiling the sections in 0.01 M citrate-buffered solution (pH 7.6) in a microwave oven (750 W, 25 min); and for SOD1 and pTDP-43 immunohistochemistry by autoclaving (121 °C, 20 min) in 0.01 M citrate buffer solution (pH 6.0).

As antibody controls, the primary antisera were either omitted or were replaced with normal rabbit or mouse serum. The preparations were examined by light microscopy.

The nomenclature used for the anatomical areas was basically that of "The Human Central Nervous System" [4], and for the detailed areas in the brain stem, that of "Cytoarchitecture of the Human Brain Stem" [5].

Patients

Seven Japanese sporadic and familial ALS patients were examined neuropathologically. All patients were receiving positive

Table 2. Pathological features of various anatomical structures.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Visual pathway							
Retina	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
Nervus opticus	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
Tractus opticus	**O**	**O**	**O**	**O**	**O**	**O**	**O**
Corpus geniculatum laterale	**O**	N.E.	**O**	**O**	**O**	**O**	N.E.
Colliculus superior	**O**	**o**	**o**	**O**	**o**	**o**	**O**
Radiatio optica	**O**	**O**	**o**	**O**	**O**	**O**	**O**
Area striata	**O**	**O**	**O**	**O**	**O**	N.E.	**O**
Olfactory (limbic) pathway							
Epithelium olfactorium	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
Bulbus olfactorius	N.E.	**O**	**O**	N.E.	**O**	N.E.	N.E.
Amygdala	**O**	N.E.	**O**	**O**	**O**	**O**	**O**
Hippocampus	**O**	**O**	**O**	**O**	**O**	**O**	**O**
Gyrus parahippocampalis	**O**	**o**	**O**	**O**	**O**	**O**	**O**
Ggyrus cinguli	**o**	**o**	**O**	**o**	**O**	**O**	**O**
Corpus mamillare	**O**	N.E.	**O**	**O**	**O**	**O**	N.E.
Fornix	**O**	X	**O**	**O**	**O**	**O**	**O**
Nucl. anterior thalami	**O**	**o**	**O**	**O**	**o**	**O**	**O**
Lobus frontalis	**o**	**o**	**O**	**o**	**O**	**O**	**O**
General sensory pathway (trigeminal and spinal)							
Meissner's/pacinian corpuscles	N.E./N.E.	N.E./N.E.	N.E./N.E.	N.E./N.E.	N.E./N.E.	N.E./N.E.	N.E./N.E.
Ganglion trigeminale/ganglion spinale	N.E./N.E.	N.E./N.E.	N.E./N.E.	N.E./N.E.	N.E./N.E.	N.E./N.E.	N.E./N.E.
Radix sensorius nervi trigemini/ radix dorsalis nervi spinalis	N.E./**O**	N.E./**O**	N.E./N.E.	N.E./N.E.	N.E./N.E.	N.E./N.E.	**O**/N.E.
/Nucl. proprius	/**O**	/**O**	/X	/X	/X	/**o**	/**O**
/Tractus spinothalamicus/tractus spinoreticularis/funiculus posterior	/X/X/**o**	/X/X/**o**	/X/X/X	/X/X/X	/X/X/X	/**o**/**o**/**o**	/**o**/**o**/**O**
Nucl. sensorius principalis nervi trigemini/ nucl. spinalis nervi trigemini/nuclei gracilis and cuneatus	X/X/N.E.	X/X/N.E.	X/X/N.E.	X/X/N.E.	X/X/N.E.	**o**/N.E./N.E.	**o**/**o**/N.E.
Lemniscus trigeminalis/lemniscus medialis	**o**/**O**	X/X	X/X	X/X	X/**o**	X/X	**o**/**o**
Nucl. VPM thalamus/nucl. VL thalamus	X/X	**O**/**O**	**O**/**O**	**o**/**o**	X/X	**o**/**o**	**O**/**O**
Gyrus postcentralis	**o**	**o**	**O**	**o**	**O**	**O**	**O**
Auditory pathway							
Tympanum	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
Cohlea	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
Nervus cochlearis	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
Nucl. cochlearis dorsalis	**O**	X	**o**	**o**	**o**	X	**o**
Corpus trapezoideum	**o**	X	X	X	X	X	**o**
Nucl. olivaris superior	**o**	X	X	X	X	X	**o**
Lemniscus lateralis	**o**	X	X	X	X	X	**o**
Colliculus inferior	**o**	X	X	X	X	**o**	N.E.
Corpus geniculatum medialis	**o**	**O**	**O**	**O**	N.E.	N.E.	X
Gyrus temporalis transversus	**o**	**o**	**O**	**O**	**O**	**O**	**O**
Other anatomical areas							
Commissura anterior	N.E.	**O**	**O**	**O**	**O**	**O**	**O**
Nucl. basalis of Meynert	**o**	**o**	**O**	**O**	**o**	**O**	**O**
Nucl. paraventricularis	**O**	**o**	**O**	**O**	**o**	**O**	N.E.
Nucl. supraopticus	**O**	**o**	**O**	**O**	**o**	**O**	**O**

O (large circle) = well preserved; **o** (small circle) = slight loss of neurons or myelinated fibers; X = severe loss of neurons or myelinated fibers; N.E. = not examined; Nucl. = Nucleus. The nomenclature used for the anatomical areas was basically that of "The Human Central Nervous System" [4], and for the detailed areas in the brain stem, that of "Cytoarchitecture of the Human Brain Stem" [5].

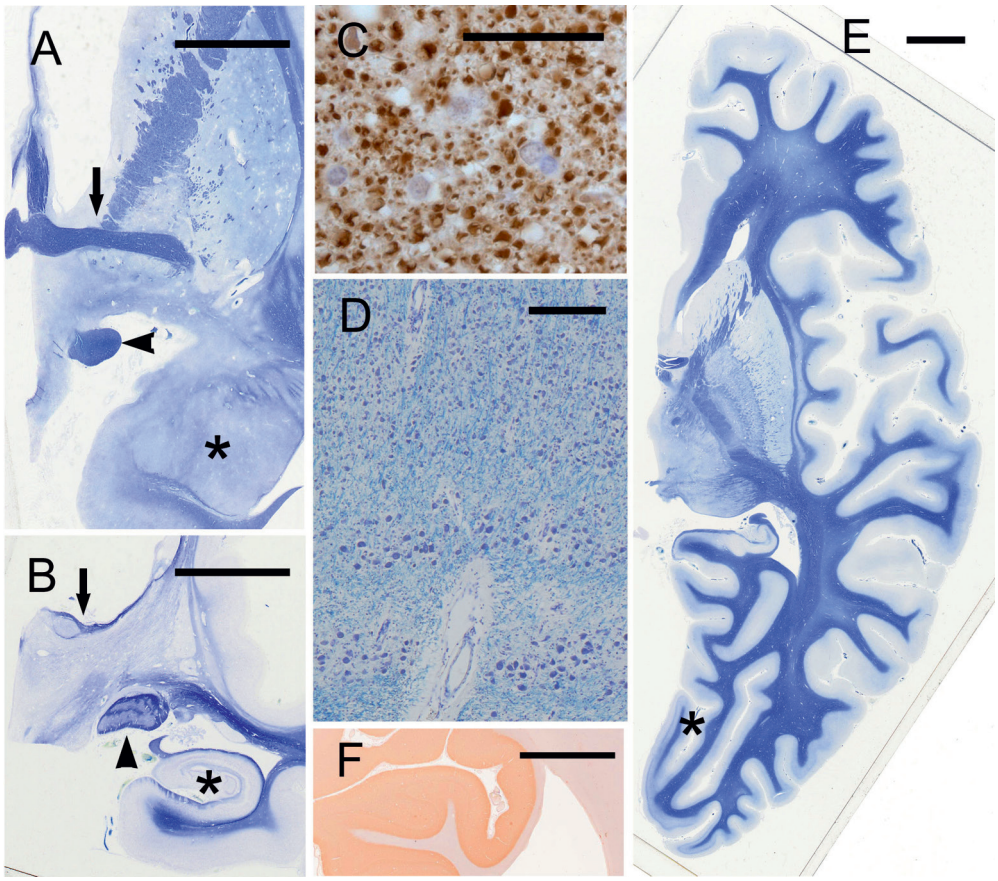

Figure 1. A: Quite well preserved tractus opticus (arrow head), commissura anterior (arrow), and amygdala (asterisk) in patient 4. B: Preserved corpus geniculatum laterale (arrow head) and hippocampus (asterisk), but severely deteriorated thalamus (arrow) in patient 6. C: Well preserved axons in the tractus opticus in patient 3 (immunohistochemistry for phosphorylated neurofilament). D: Preserved geniculatum laterale in patient 3. E: Well preserved radiatio optica and area striata (asteriks) in patient 7. F: Preserved presynaptic density in area stiata in patient 1 (immunohistochemistry for synaptophysin). A, B, D and E: Klüver-Barrera preparation. Bars: 10 mm in A, B, E and F; 20 μm in D, and 50 μm in C.

pressure ventilation support via a tracheostomy (TPPV). The patients were 4 males and 3 females, aged between 40 and 69 years at the time of death. The ages of the patients at disease onset had ranged from 13 to 64 years (mean 46.1 years) and the duration of the illness had ranged from 5 to 27 years (mean 11.7 years). The duration of respirator use had ranged from 3 to 26 years. Four patients had a genetic background for ALS (patients 1, 3, 5, and 6), and 3 had sporadic ALS. All patients had been in a TLS, i.e., stage V of Hayashi et al. [6], before death. None of the patients had shown dementia before entering the TLS (Table 1). Some of the clinical and neuropathological findings of these patients have been reported previously [6, 7, 8, 9, 10]. Informed consent was obtained from each family member. This study was performed in accordance with the provisions of the Declaration of Helsinki (1995), and was approved by the Ethics Committees of Shinshu University (No. 1772), and Tokyo Metropolitan Neurological Hospital.

Results

All patients showed evident degeneration of the anterior and lateral tractus corticospinalis, and severe loss of Betz cells and neurons in the cornu anterioris spinalis, nuclei facialis, and hypoglossi. The tegmentum of the brain stem, thalamus, and globus pallidus showed marked atrophy in every patient. In addition, patient 1 showed marked degeneration of the somatosensory cortices, frontal and parietal cortices, frontal white matter, striatum, subthalamic nucleus, substantia nigra, and the Clarke's nucleus. Posterior column was relatively preserved, but the other white matter was lost in the spinal

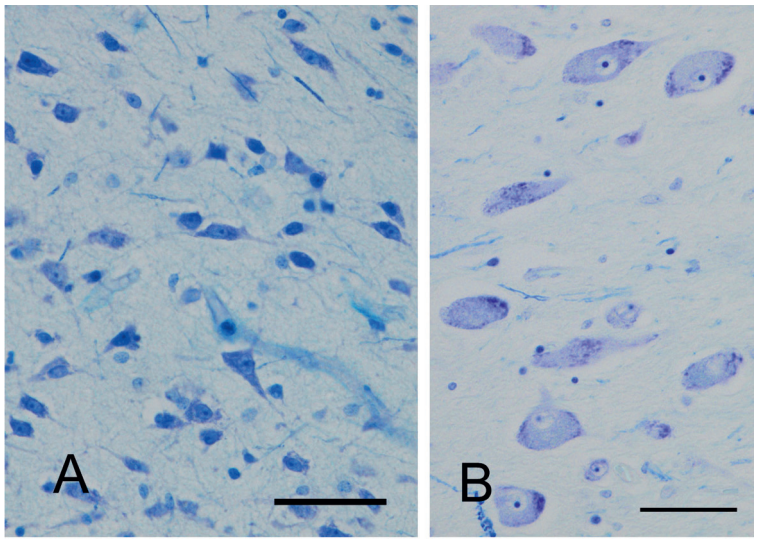

Figure 2. Well preserved bulbus olfactorius (A: patient 3) and nucleus basalis of Meynert (B: patient 7). A, B: Klüver-Barrera preparation. Bars: 50 μm.

cord. FUS-immunoreactive (ir) neuronal cytoplasmic inclusions (NCIs) were many in the frontal and motor cortices. Some FUS-ir glial inclusions were observed in the cerebral cortex and the white matter. Patient 1 did not show dementia in the clinical course, but the neuropathological subtype should be FTLD-FUS [10]. In patient 2, the substantia nigra, subthalamic nucleus, dentate nucleus, spinal anterolateral funiculus, Clarke's nucleus, dorsal spinocerebellar tract, posterior funiculus, and subiculum were degenerated. Scattered TDP-43-immunopositive glial inclusions were found. TPD-43 pathology is classified as type 2 [11]. Gene analysis including C9orf72 repeat was not performed. The patient did not show dementia in the clinical course, but the neuropathological subtype should be FTLD-TDP.

TPD-43 pathology of patients 4 and 7 is classified as type 1 [11]. Recent gene analysis of patient 6 revealed that K510M mutation in the FUS [9].

Among these patients, the tractus opticus, corpus geniculatum laterale, colliculus superior, radiatio opticus, and area striata were quite well preserved (Table 2) (Figure 1), though the retina and nervus opticus were not available. With regard to the olfactory pathway, the epithelium olfactorium was not examined, while mitral cells of the bulbus olfactorius, amygdala, hippocampus, gyrus parahippocampalis, gyrus cinguli, corpus mammillare, fornix and nucleus anterior thalami, and lobus frontalis were well preserved (Table 2) (Figure 1, 2). In addition, the commissura anterior, nucleus basalis of Meynert, nucleus paraventricularis, and nucleus supraopticus were well preserved (Table 2) (Figure 1, 2).

On the other hand, in the somatosensory system, the funiculus posterior in the spinal cord, nucleus spinalis nervi trigemini sensibilis principalis, and lemniscus medialis and lemniscus trigeminalis in the brain stem showed severe degeneration (Table 2) (Figure 3), though relatively well preservation was examined in the thalamus and gyrus postcentralis. In the auditory pathway as well, the nucleus trapezoideus, nucleus olivaris superior, lemniscus lateralis, and colliculus inferior were deteriorated in most of the patients (Table 2) (Figure 3), though nucleus cochlealis dorsalis, corpus geniculatum medialis and gyrus temporalis transversus were preserved relatively well. In the gustatory pathway, the nucleus parabrachialis medialis and tractus trigeminothalamicus dorsalis were degenerated in 6 of the 7 patients (Table 2) (Figure 3).

Discussion

As of 2004, 3% of ALS patients in France were reported to be receiving TPPV support [12], compared with 28.4% in Japan, among whom 13% were in a TLS [13].

Hayashi et al. [6] recently proposed a staging system for classification of communicative ability in ALS patients, stage I: communicate in sentences; stage II: communicate with one word answers only; stage III: communicate with nonverbal yes/no response only; stage IV: cannot communicate occasionally due to uncertain yes/no responses; stage V: cannot communicate by any means, i.e., a TLS. Clinical analysis showed that patients who reached stage V had begun to use the ventilator significantly earlier than patients in whom the final stage was IV or less. All patients in stage V (TLS) were under TPPV support. In addition, such patients frequently had a family history of ALS [6, 7, 8]. All the patients examined in the present study had stage V ALS.

Preservation of visual evoked potential (VEP) has been reported in ALS patients

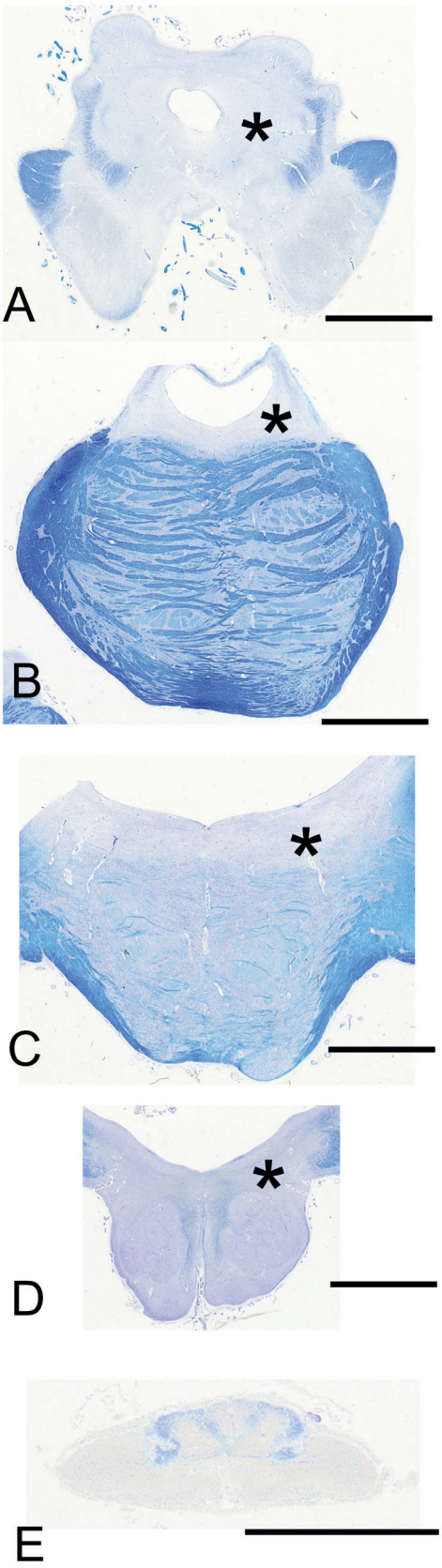

Figure 3. Severe deterioration of the formatio reticularis (asterisks) in the brain stem. A: Marked degeneration of the tectum, tegmentum and medial and central portions of the midbrain in patient 1. B and C: Severe atrophy of the tegmentum of the pons in patient 3. D: Marked degeneration of the formatio reticularis and pyramis in the medulla oblongata of patient 2. E: Deteriolation of the funiculus gracilis and the middle root zone in the funiculus cuneatus in addition to complete degeneration of the funiculi laterale, anterolaterale, and anterior in the cervical cord in patient 5. A – E: Klüver-Barrera preparation. Bars: 10 mm.

[14, 15], and Lim et al. [16] have examined steady-state VEP (SSVEP) in an ALS patient with impaired oculomotor function. Remarkable preservation of the visual pathway in ALS patients in a TLS elucidated in the present study may indicate that photic stimulation reaches the area striata. Thus, communication that exploits visual acuity is considered to be the best approach for such patients. The visual function of patients with ALS in a TLS should therefore be examined, and tractography by magnetic resonance imaging (MRI) [17] may disclose well preservation of the visual pathway in patients of ALS in TLS.

In addition, the present study revealed preservation of the olfactory pathway, suggesting that olfactory function was also retained.

It has been reported that median nerve somatosensory evoked potentials (SEP) decrease in accordance with muscle weakness in ALS patients [18], but no study has investigated such patients in a TLS. The present and previous results indicate that the somatosensory and auditory pathways are severely damaged in patients in TLS, and thus somatosensory and auditory function would appear to be compromised.

It has also been reported that the reticular formation of the brain stem is severely degenerated in patients with ALS in a TLS [7, 8]. As the reticular formation plays a very important role as the reticular activating system, its degeneration would indicate difficulty with information processing in the cerebrum. However, preservation of the limbic area including the hippocampus, gyrus parahippocampalis, amygdala, and nucleus basalis of Meynert indicate the probable preservation of memory acuity; in addition, preservation of cognitive function has been suggested in patients with ALS in a TLS through the use of optical topography [19].

Preservation of the nuclei paraventricularis and supraopticus in patients with ALS

in a TLS may represent a normal degree of secretion of antidiuretic hormone. The nucleus suprachiasmaticus in the present patients was not available for study. Examination of the circadian rhythm of such patients might shed new light on these aspects in the TLS associated with ALS.

The present paper reports a novel finding of the marked preservation of the optic and olfactory system in patients in the TLS due to ALS. The findings indicate a preservation of optic and olfactory input systems to the brain of the patients. As for integrating system in those patients, the number of neurons in the cerebral cortex was relatively preserved, and a paper reported a possibility to make a communication with those patients by classifying electroencephalogram data [1]. As for the output system, there have been no reports on the electromyography in the patients in TLS-ALS. Since the course of developing TLS is usually gradual, future advance of brain machine interface (BMI) may elongate the period of communication with the patients, and even in the end-stage, some BMI may enable to communicate with the patients and move extremities by detecting weak signals of output from the patients.

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Conflict of interest

The authors report no conflict of interest.

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Clinical short communication

ALS patients with ability to communicate after long-term mechanical ventilation have confined degeneration to the motor neuron system

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Neuropathological findings

ABSTRACT

Objective: To clarify the position in the amyotrophic lateral sclerosis (ALS) spectrum, of a subgroup of patients who maintained the ability to communicate after long-term mechanical ventilation (LTMV) by tracheostomy.
Methods: We undertook a clinicopathological investigation of sporadic ALS in three patients who maintained the ability to communicate after approximately 30-year survival on LTMV by tracheostomy.
Results: The age of onset and duration of disease was 48 years and 31 years in patient 1, 55 years and 29 years in patient 2, and 31 years and 33 years in patient 3, respectively. Each patient displayed slow disease progression. In all patients, both upper and lower motor neurons were markedly degenerated, while other neuronal systems and the brainstem tegmentum were spared. A few normal-looking motor neurons remained in the anterior horn of the spinal cord. There were no TAR DNA-binding protein 43-immunoreactive inclusions in the lower motor neurons in any patient and only occasional inclusions in the cerebral cortex of one patient.
Conclusion: The clinicopathological findings of these three patients suggest that there is a distinct subgroup of ALS patients characterized by the above-mentioned features.

1. Introduction

Most patients with amyotrophic lateral sclerosis (ALS) will die from respiratory failure 2–4 years after the onset of symptoms unless treated by mechanical ventilation [13]. In patients with ALS, tracheostomy invasive ventilation (TIV) may extend survival for years [3,4,14]. The use of TIV may depend on social, cultural, religious, or economic factors [1,5]. Often ALS patients and their close relatives decide not to use TIV due to the fear of progressing into a totally locked-in state [6], with loss of all voluntary movement, including eye movement [1,4,9]. Between 8% and 17.2% of ALS patients using TIV develop a totally locked-in state [9,10]. However, some ALS patients maintain their ability to communicate after long-term mechanical ventilation (LTMV) by tracheostomy [8,15]. The position that the ALS in this patient subgroup occupies in the disease spectrum has not previously been described.

2. Patients and methods

To clarify the position of ALS in this patient subgroup in the disease spectrum, we conducted a clinicopathological investigation of sporadic ALS cases in three Japanese patients who maintained the ability to communicate after approximately 30-year survival on mechanical ventilation by tracheostomy.

3. Results

3.1. Clinical features

Table 1 shows the clinical findings of the three patients [16]. Nerve conduction studies at the time of diagnosis in patient 1 revealed reduced motor response of the median nerve and normal sensory nerve potentials. Needle electromyogram (EMG) showed active and chronic denervation changes in the upper and lower extremity muscles, supporting the diagnosis of ALS. EMG records for patients 2 and 3 could not be found and were not available. Patients 1 and 2 showed weakness and muscle atrophy of distal upper extremities

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Table 1
Summary of clinical findings in three cases with surviving on long-term mechanical ventilation by tracheostomy amyotrophic lateral sclerosis.

Case number	1	2	3
Sex	Man	Man	Man
Age of onset (years)	48	55	30
Disease duration (years)	28	29	33
Initial symptom	Right hand weakness	Left hand weakness	Right hand weakness
Tendon reflexes	Increased	Increased	Increased
Progression rate of ALSFRS-R at time of diagnosis[a]	0	0	0
Duration from the onset (years)			
To tracheostomy	8	14	6
To mechanical ventilation	13: sometimes, 25: always	14	6
To total quadriplegia	26	Eventually, knee joints move a little	11
Eye movement	Preserved	Mild vertical ophthalmoparesis	Preserved
Dementia	–	–[b]	–
Cause of death	Malignant lymphoma	Sudden death	Acute circulatory failure

[a] Progression rate of ALSFRS-R was calculated as: (48-ALSFRS-R at "time of diagnosis") / duration from onset to diagnosis (month) [16].
[b] Delirium occurred temporarily at the time of 28 years from the onset.

and then showed spasticity of lower extremities 7 months and 9 years from disease onset, respectively. Their spasticity continued after tracheostomy and LTMV. Therefore, the predominant symptoms were considered to be upper motor neuron disorder and a slowly progressing disease course in patients 1 and 2. Patient 3 showed hyperreflexia and the ALS progressed earlier than in the other patients and he became quadriplegic. After LTMV by tracheostomy for patient 3, progression of symptoms appeared to stop because his eye and oral movements were functioning well until his death. All three patients were discharged to their home with a home support system after tracheostomy and LTMV. They could communicate via eye movements until their end of life, and could read newspapers and books.

3.2. Neuropathological features

Before fixation, the brain of patient 1 weighed 1190 g, patient 2 1030 g, and patient 3 1290 g. Mild frontal lobe atrophy was seen in patient 2; however, neuronal loss in the cortex and gliosis of the white matter was not observed. All patients showed motor neuron system-confined degeneration, mild brainstem atrophy with preserved tegmental fibers and marked spinal cord atrophy (Fig. 1A–E). There was moderate loss of motor neurons in the anterior horn of the spinal cord (Fig. 1F) and brainstem motor nuclei, with the exception of ocular nuclei, in patients 1 and 2, and the loss was marked in patient 3. However, in all patients, a few normal-looking motor neurons remained in the anterior horn of the spinal cord (Fig. 1G, H). Although some spheroids

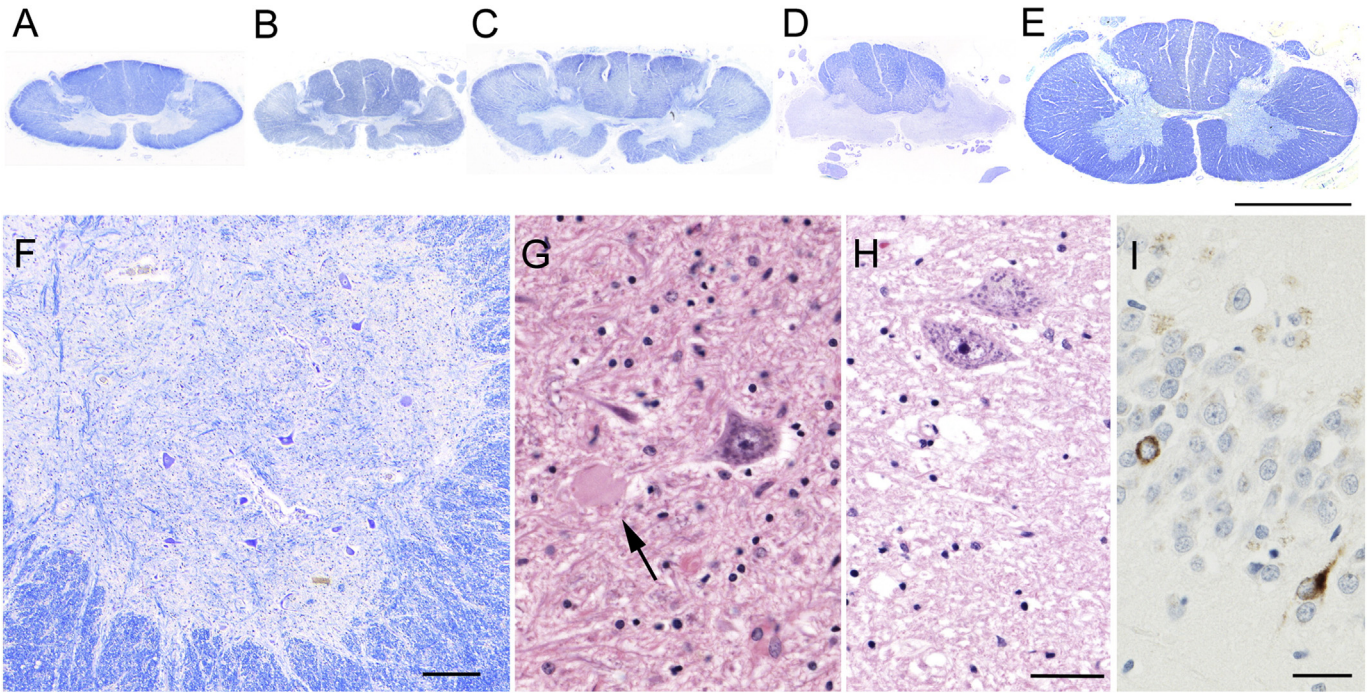

Fig. 1. A–E: Cervical cord at the same magnification. A: patient 1, B: patient 2, C: patient 3, D: A 66-year-old patient with a 13-year-5-month history of amyotrophic lateral sclerosis (ALS) who progressed to a totally locked-in state, E: A 72-year-old patient with Parkinson disease. The cervical cord of patient 1–3 (A–C) shows marked atrophy, which is similar to that in the patient with ALS who progressed to a totally locked-in state (D). However, degeneration of the anterior horn, lateral and anterior column is milder in A-C than D, despite the longer disease duration in patient 1–3 (bar = 5 mm, Klüver–Barrera staining). F–I: Anterior horn of the fifth lumbar cord. F: A small number of motor neurons remained (patient 1, Klüver-Barrera staining, bar = 200 μm). G and H: Normal-looking motor neurons and a spheroid (arrow) are observed with mild fibrillary astroglial proliferation. However, no hypertrophic astrocytes, microglia, or macrophages are seen (G: patient 1, H: patient 3, Hematoxylin and eosin staining, bar = 50 μm). I: TAR DNA-binding protein 43-immunoreactive neuronal cytoplasmic inclusions are seen in the granular cells of the hippocampal dentate gyrus (patient 3, bar = 20 μm).

were seen (Fig. 1G), no hypertrophic astrocytes were observed (Fig. 1 G, H). Furthermore, no microglia or macrophages were seen in the lower motor neuron system or in the upper motor neuron system by immunostaining with CD-68 (KP-1) (1:400, Novocastra). There were no TAR DNA-binding protein 43- (TDP-43) (pS409/410-1 1:3000, CosmoBio) immunoreactive inclusions or Bunina bodies in the lower motor neurons in any patient. However, in patient 3, TDP-43-immunoreactive neuronal cytoplasmic inclusions (NCI) were found in the cerebral cortex, hippocampal dentate granule neurons (Fig. 1I), reticular formation of the medulla oblongata, and inferior olivary nuclei. There were no fused in sarcoma (FUS) or superoxide dismutase 1 (SOD1) immunereactive inclusions seen in any patient.

3.3. Western blotting of TDP-43

Sarkosyl-insoluble TDP-43 prepared from frozen brain tissues of the patient 2 was investigated by comparing with that in a 78-year-old patient with a two-year history of ALS. No phosphorylated TDP-43 was detected in the patient 2 (Fig. 2). Due to the lack of available frozen tissue from patients 1 and 3, this analysis could only be performed on tissue from patient 2.

4. Discussion

Three patients with ALS showed both upper and lower motor neuron disorder, without ophthalmoplegia, autonomic disorder, cerebellar signs, extrapyramidal signs, or dementia, and all survived on LTMV by tracheostomy for about 30 years. Prominent upper motor neuron disorder, which is characterized by spasticity in the lower extremities and weakness in the upper extremities, was evident in the early state of disease in patients 1 and 2. These features are different from the features of primary lateral sclerosis and may be features of long survival with ALS [17]. The disease duration was extremely long among patients reported with ALS on LTMV by tracheostomy [3,4,14]. Survival studies indicate that advantageous criteria for long-term survival after tracheostomy were age ≤ 65 years at the time of TIV initiation [14] and patients with home care [4,14]. These three patients met these criteria: all three patients lived at home and two of them received their tracheostomy at <65 years of age. However, it is important to emphasize that the time from onset until the initiation of LTMV by tracheostomy and to become totally quadriplegic was relatively long in all patients. Previously we reported that patients with rapidly progressing ALS before initiation of TIV might progress further to a totally locked-in state (communication stage V) [7]. Furthermore, Nakayama et al. [10] clarified that TIV, impaired oculomotor movement, and total quadriplegia are predictors of severe communication impairment. Comparing the disease progression in the present patients with that in the reported patients who progress to a totally locked-in state, suggests that the rate of disease progression before TIV might predict the long-term prognosis for communication impairment after LTMV by tracheostomy. Slow disease progression before TIV in ALS patients might predict a better long-term prognosis for communication impairment. Among the present patients with a better prognosis, the ALS progressed very slowly; the ALS in the third patient apparently stopped progressing at all. The clinical course of the present patients suggests that they fall into a subgroup of patients with slow ALS progression who maintain the ability to communicate after long-term survival on mechanical ventilation.

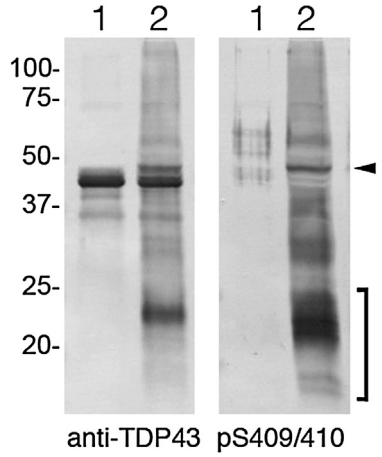

Fig. 2. Western blot analysis of the sarkosyl-insoluble fractions extracted from cryopreserved precenetral tissue. Anti-TAR DNA-binding protein 43- (TDP-43) antibody (Proteintech, 10782-2-AP rabbit polyclonal) detected a strong band for full length unphosphorylated normal TDP-43 in patient 2 (lane 1), but some abnormal TDP-43 bands at 45kD (arrow head), ~23 kDa fragments and smears in a 78-year-old patient with a two-year history of ALS (lane 2). Analysis with anti-phospho TDP-43 antibody (pS409/410, Cosmobio, pS409/410-1 rabbit polyclonal) detected the phosphorylated forms of full length TDP-43 at 45 kDa, C-terminal fragment (CTFs) at 18–25 kDa and smears, type B CTF banding pattern [18] in the two-year history ALS patient (lane 2). In contrast, no such band was detected in patient 2 (lane 1). Analysis with anti-tau antibody (T46, Thermo, mouse monoclonal) showed no tau accumulation in patients 2 (data not shown).

Neuropathologically, confinement of degeneration to the motor neuron system in the present patients appears to correspond to their clinical features, which were similar to those in usual cases of ALS in terms of site of onset and symptoms. These features were the same as in reported cases [8,15] and indicated that there are ALS cases that do not progress beyond the motor neuron system despite extremely prolonged survival with TIV by tracheostomy. The histopathological characteristics, in which motor neurons in the anterior horn of the spinal cord and spheroids remained normal looking, were usually observed in ALS cases at an earlier stage [2]. The absence of macrophages and hypertrophic astrocytes indicates that their lesions were not active [15]. Therefore, neuropathologically the ALS in these patients showed slow progression or likely stopped progressing. Thus, the ALS was different from usual cases of ALS. Further, Bunina bodies and TDP-43-immunoreactive inclusions, which are characteristic of sporadic ALS, were not observed in the motor neurons. Western blot analysis of TDP-43 in precenetral tissue from patient 2 revealed that accumulation of abnormal forms of TDP-43 proteins were low or below the detection limit. However, the distribution and appearance of TDP-43-immunoreactive NCI in the third patient was the same as that seen in the non-motor areas of ALS patients [11]. Therefore, at least the third patient might have had TDP-43 pathology. The degree of TDP-43-immunoreactive NCI infiltration is milder in patients with longer disease duration [12]. There is a possibility that we could not find TDP-43/ubiquitin immunoreactive NCIs because they were so rare.

It is important to note some limitations of this study. First, the study is a small sample. Second, not all clinical measures were available because of the long duration of disease, and tissue samples from all patients could not be available. However, it is rare for long survival patients, who stay mainly at home, to provide autopsy studies. Nonetheless, the available clinicopathological findings in these three patients suggest that there is a distinct subgroup of ALS patients. This ALS patient subgroup maintained the ability to communicate after survival on LTMV by tracheostomy.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

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NEUROPATHOLOGY

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Case Report

A Japanese familial ALS patient with autonomic failure and a p.Cys146Arg mutation in the gene for SOD1 (*SOD1*)

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We describe a Japanese man with familial amyotrophic lateral sclerosis (ALS) associated with a p.Cys146Arg mutation in the copper/zinc superoxide dismutase gene (*SOD1*). The patient developed bulbar signs followed by rapidly progressive limb muscle weakness. The prominent clinical feature was orthostatic hypotension due to autonomic failure, which occurred after he underwent tracheostomy 1 year and 3 months after the onset. Thereafter, he required mechanical ventilation and progressed to communication stage V (totally locked-in state) 7 years after the onset. Neuropathology showed ALS with posterior column degeneration and multiple system degeneration. Severe neuronal loss in the intermediolateral nucleus was also observed. Two previously reported cases of ALS patients with autonomic failure showed severe neuronal loss in the intermediolateral nucleus in addition to degeneration of the motor neurons. Thus, autonomic failure due to neuronal loss in the intermediolateral nucleus could present in patients with ALS associated with certain mutations in *SOD1*.

Key words: autonomic failure, familial ALS, orthostatic hypotension, *SOD1*, p.Cys146Arg.

INTRODUCTION

The main feature of autonomic dysfunction in patients with amyotrophic lateral sclerosis (ALS) has been reported to be sympathetic hyperactivity and sympathovagal imbalance.[1–3] Autonomic failure, including severe orthostatic hypotension, does not usually emerge in ALS, even during advanced stages with ventilator use. Here we describe an autopsy case of a familial ALS patient who presented with autonomic failure from a relatively early stage of the disease. The patient had a p.Cys146Arg mutation in the copper/zinc superoxide dismutase gene (*SOD1*). The clinical course of ALS in patients with this mutation has been reported in one family; however, neither autonomic nervous dysfunction nor pathological features had been described.[4] The present study is the first clinicopathological report related to this mutation. We also compared previously reported cases of ALS with autonomic failure[5,6] with the present case and discuss the pathomechanism of autonomic failure in ALS.

CLINICAL SUMMARY

A 58-year-old Japanese man (Subject III-8 in Fig. 1) developed hoarseness, followed by dysarthria. His younger sister, niece and three cousins had also been diagnosed as having ALS (Fig. 1). His younger sister (Subject III-11 in Fig. 1) developed bulbar palsy at 38 years of age and died without respiratory support at 41 years of age. His niece (Subject IV-9 in Fig. 1) developed right leg weakness at 32 years of age and received respiratory support beginning 1 year and 2 months after the onset; her symptoms progressed without upper motor neuron issues and she currently lives free from autonomic failure. Two cousins (Subjects III-3, 4 in Fig. 1) developed leg weakness in the third decade and died in the fourth decade of life. Another cousin (Subject III-14 in Fig. 1) developed bulbar palsy in the sixth decade of life and died at 65 years of age. Six months after the onset of the disease in our patient, neurological examination showed mild bulbar palsy, mild muscle weakness, and muscle atrophy affecting the face and all extremities, without hyperreflexia. He had no sensory disturbance, cerebellar ataxia, cognitive dysfunction or abnormal blood pressure

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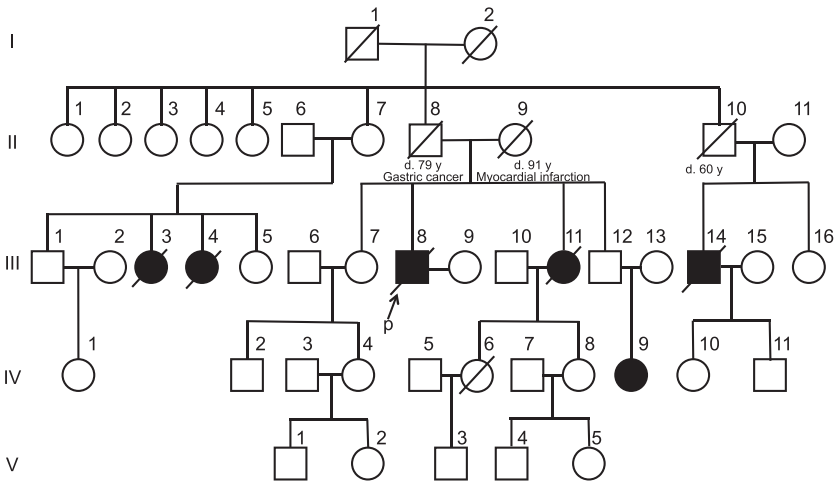

Fig. 1 Pedigree of the family. The arrow indicates the proband. Affected individuals are represented by solid black squares and circles.

fluctuation suggesting sympathetic hyperactivity. Nerve conduction studies showed normal conduction velocities in both sensory and motor nerves. Needle electromyography showed active denervation potentials in the muscles of the upper and lower extremities and the thoracic paraspinal muscles. MRI studies of the brain and the cervical spine were normal. He was diagnosed as having a progressive muscular atrophy variant of familial ALS. He developed exertional dyspnea beginning 9 months after the onset, then underwent a tracheostomy and percutaneous endoscopic gastrostomy 1 year and 3 months after the onset. After the tracheostomy, syncope due to orthostatic hypotension frequently occurred. Mechanical ventilation was started 2 years and 3 months after the onset, and a head-up tilt test showed orthostatic hypotension with a systolic blood pressure reduction of 40 mmHg (from 128/93 mmHg to 85/65 mmHg) without compensatory tachycardia and faintness. The plasma norepinephrine response was poor, suggesting vasomotor sympathetic efferent dysfunction. These findings indicated that the patient was experiencing autonomic failure. Urodynamic analysis showed an uninhibited bladder. Coefficient of variation of R-R intervals on electrocardiogram showed low value at 1.0%. Clinically, no sweating abnormality was observed. He became a total quadriplegic without upper motor neuron signs 3 years and 9 months after the onset of the disease. Thereafter he exhibited progressive external ophthalmoplegia, resulting in a complete loss of all voluntary movement, representing a totally locked-in state[7] or stage V of the communication ability stage,[8] 7 years after onset. He died of pneumonia at 68 years of age. An autopsy was conducted 6 h after death.

Genetic analysis

DNA was extracted from the patient's leukocytes using a conventional method with informed consent. All exons and exon–intron boundaries of *SOD1* were examined by directly sequencing PCR products. Genetic analysis revealed a heterozygous T-to-C transition at cDNA position 436, resulting in a Cys146Arg missense mutation within exon 5.

PATHOLOGICAL FINDINGS

Brain weight was 1200 g prior to formalin fixation. Macroscopically, the spinal cord and brainstem showed marked atrophy while the frontal and temporal lobes showed mild atrophy. The globus pallidus, subthalamic nucleus and substantia nigra were brownish in color (Fig. 2A). The other structures in the cerebrum and the cerebellum appeared normal. Microscopically, the anterior horn of the spinal cord (Fig. 2 M) and all motor nuclei including the oculomotor nucleus of the brainstem showed severe neuronal loss and gliosis. In the very few remaining lower motor neurons, no Bunina bodies, Lewy body-like hyaline inclusions, phosphorylated transactive response DNA-binding protein 43 kDa (TDP-43) (pS409/410; CosmoBio, Tokyo, Japan; 1:5000), neurofilament (DAKO, Tokyo, Japan; 1:500), SOD1 (Proteintech, Tokyo, Japan; 1:100) immunoreactive neuronal cytoplasmic inclusions were found. The primary motor cortex showed a marked loss of Betz cells and pyramidal neurons with gliosis, and the corticospinal tract showed marked fiber loss (Fig. 2H). In the spinal cord, in addition to the corticospinal tract, the dorsal column, posterior spinocerebellar tract and antero-lateral funiculus also showed marked fiber loss (Fig. 2 K,L). Neurons within Clarke's nucleus (Fig. 2 L,O) as well as in the intermediolateral nucleus (Fig. 2 L,N) were markedly decreased with gliosis. Dorsal root ganglion showed slight neuronal loss with a few Nageotte's nodules. In the brainstem tegmentum, almost all nuclei and fibers including the following structures showed marked loss with gliosis: neurons of the accessory cuneate nucleus, dorsal motor nucleus of the vagus, solitary tract nucleus, superior colliculus and locus ceruleus, and reticular formation, as well as fibers of the

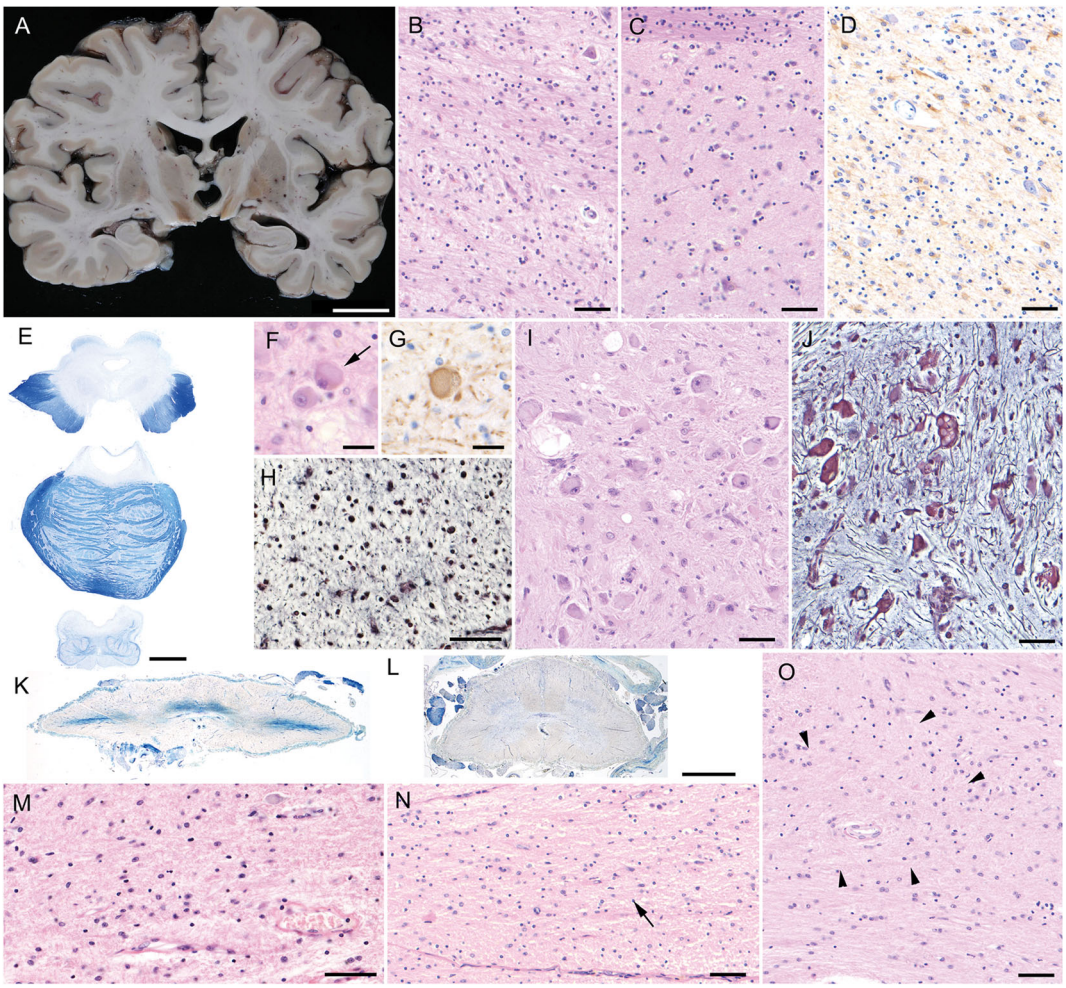

Fig. 2 Neuropathological findings. (A) In a coronal section, the subthalamic nucleus and globus pallidus are brownish in color, and the frontal lobe shows mild atrophy. Bar = 2 cm. (B) Internal segment of the globus pallidus shows moderate neuronal loss and gliosis. (C) Putamen shows slight gliosis. (D) Subthalamic nucleus shows moderate neuronal loss and gliosis with GFAP (Novocastra Laboratories, 1:500) immunostaining. (E) The midbrain and medulla oblongata shows marked atrophy and the pons shows moderate atrophy. Neurons and fibers of the tegmentum and corticospinal tract are severely reduced. Bar = 5 mm. (F, G) Neurofilamentous conglomerate inclusions (F; HE) reveal staining for neurofilament (G) in pontine nuclei. (H) Both large and small fibers are lost in the medullary pyramid with Bodian staining. (I, J) Vacuolar degenerated neuron and gemistocytic astrocytes are observed in the inferior oivary hypertrophy (I; HE, J; Bodian). Marked atrophy and fiber loss are observed in the spinal cord at the seventh cervical cord (K) and 12th thoracic cord (L). Bar = 2 mm. (M) Anterior horn of the seventh cervical cord shows marked neuronal loss with gliosis. (N, O) Marked neuronal loss is seen in the intermediolateral nucleus (arrow, N) and Clarke's nucleus (surrounded by arrowheads, O) at the 12th thoracic cord. (B, C, F, I, M-O) HE staining. (E, K, L) KB staining. (F, G) Bar = 20 μm, (B-D, H-J, M-O) Bar = 50 μm.

central tegmental tract and superior cerebellar peduncles (Fig. 2E). Neurons of the pontine nuclei were atrophied, although the total number was preserved. Several neurons in the inferior olivary nucleus showed vacuolar degeneration with gemistocytic astrocytes known as inferior olivary hypertrophy[9] (Fig. 2I, J). The middle and inferior cerebellar peduncle and olivocerebellar fibers were relatively well preserved. The substantia nigra showed severe neuronal loss with gliosis, globus pallidus (Fig. 2B), and subthalamic nucleus (Fig. 2D) showed moderate neuronal loss with gliosis, and the caudate nucleus and the putamen (Fig. 2C) showed slight gliosis. There were no SOD1-immunoreactive structures in these areas. However, the neurofilament immunoreactive neurons were scattered in the degenerated areas (Fig. 2F, G). Neurons of the hippocampus and amygdala were preserved. Neurofibrillary tangles (NFTs) were observed in the entorhinal cortex and the hippocampus (Braak NFT stage II) using anti-phosphorylation-dependent tau (AT8: Innogenetics, Gent, Belgium; 1:5000) immunostaining. There were only a few senile plaques with Methenamin Bodian staining. In the cerebellum, neurons of the dentate nucleus and fibers of the hilum of the dentate nucleus showed a marked loss in addition to a mild decrease in the number of Purkinje cells, especially in the vermis.

There were no Lewy bodies and glial cytoplasmic inclusions using phosphorylated α-synuclein (WAKO, Tokyo, Japan, 1:2000) immunostaining.

DISCUSSION

We herein reported an autopsy case of Japanese familial ALS with a p.Cys146Arg mutation in *SOD1*. Characteristic clinical features of this patient included bulbar onset, predominant lower motor neuron signs and rapid progression to communication stage V;[8] however, the most distinctive characteristic was orthostatic hypotension due to autonomic failure shortly before tracheostomy. Pathologically, this patient had lesions typical of ALS with posterior column involvement; this included both upper and lower motor neurons, Clarke's column, the spinocerebellar tract, and the posterior column of the spinal cord. In addition, the ALS findings also showed multiple system degeneration, such as degeneration of the brainstem tegmentum, globus pallidus, subthalamic nucleus and substantia nigra. Severe neuronal loss in the intermediolateral nucleus was also observed.

The clinical courses of affected members of this family and reported two siblings with a p.Cys146Arg mutation in *SOD1*[4] were variable as follows. (i) Although the majority of familial ALS patients with a mutation in *SOD1* have onset in the limbs,[10] four of eight patients with p.Cys146Arg mutation developed from bulbar palsy and the other four patients developed from leg weakness. (ii) The age at onset varied from 32 years old to sixth decades of life. (iii) Three patients progressed rapidly to die or underwent tracheostomy invasive ventilation (TIV) in 2 years, in contrast to one patient who had a slowly progressed phenotype. (iv) Only our patient showed autonomic failure. These findings suggest that ALS patients with a p.Cys146Arg mutation in *SOD1* show variable clinical features and courses.

Among three reported ALS patients with a mutation in *SOD1* and autonomic dysfunction,[2,6,11] only one patient had autonomic failure.[6] This patient showed rapid disease progression and developed to communication stage V[8] 3 years after the onset, and then showed autonomic failure. Her autonomic failure developed later than in our patient, although the neuropathological findings of both the reported patient[6] and our patient were the same. These patients showed neuronal loss in the intermediolateral nucleus, which is a sympathetic preganglionic nucleus,[12] and degeneration of the brainstem tegmentum which included nuclei of the central autonomic network such as the solitary tract nucleus and the ventrolateral medulla.[12] However, these patients did not show symptoms of sympathetic hyperactivity prior to autonomic failure. Their clinical course and pathological features suggest that even in ALS patients, autonomic failure occurs when the intermediolateral nucleus has severe degeneration.

Another patient with autonomic failure and motor neuron disease[5] developed syncope caused by autonomic failure. Neuropathologically, degeneration of both the upper and lower motor neurons was reported. In addition, degeneration of the posterior column of the spinal cord was observed along with intracytoplasmic Lewy body-like hyaline inclusions and conglomerates and hyaline inclusions in the anterior horns, which are pathological hallmarks of ALS with a mutation in *SOD1* (*SOD1*-related ALS).[13] Marked neuronal loss in the intermediolateral nucleus of this patient indicates that neuron loss in the intermediolateral nucleus can occur when degeneration of the motor neurons is mild to moderate.

Previous reports have demonstrated that neurons of the intermediolateral nucleus can be preserved in *SOD1*-related ALS patients without autonomic failure.[13–15] Takahashi *et al.*[16] reported that in sporadic ALS patients on TIV, neurons of the intermediolateral nucleus were reduced by 60% at the second thoracic segment and 57% at the ninth thoracic segment. Even in very long duration ALS patients with severe degeneration of multiple systems, some neurons remained in the intermediolateral nucleus.[17] Therefore, the loss of neurons in the intermediolateral nucleus of the present patient and the two reported patients with autonomic failure was an unexpected finding.

Our patient had inferior olivary hypertrophy. Although inferior olivary hypertrophy had been reported in progressive supranuclear palsy[18] and Creutzfeldt Jakob disease[19] in addition to cerebrovascular disease,[9] it has not been reported in ALS. However, in our hospital, one other ALS patient who progressed to stage V also showed inferior olivary hypertrophy (unreported case). These findings revealed that inferior olivary hypertrophy may occur also in ALS.

In conclusion, autonomic failure due to neuronal loss in the intermediolateral nucleus could present in patients with ALS associated with some *SOD1* mutations.

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Clinicopathological characteristics of patients with amyotrophic lateral sclerosis resulting in a totally locked-in state (communication Stage V)

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Abstract

In the present study, we performed a comprehensive analysis to clarify the clinicopathological characteristics of patients with amyotrophic lateral sclerosis (ALS) that had progressed to result in a totally locked-in state (communication Stage V), in which all voluntary movements are lost and communication is impossible. In 11 patients, six had phosphorylated TAR DNA-binding protein 43 (pTDP-43)-immunoreactive (ir) neuronal cytoplasmic inclusions (NCI), two had fused in sarcoma (FUS)-ir NCI, and three had copper/zinc superoxide dismutase (SOD1)-ir NCI. The time from ALS onset to the need for tracheostomy invasive ventilation was less than 24 months in ten patients. Regardless of accumulated protein, all the patients showed common lesions in the pallido–nigro–luysian system, brainstem reticular formation, and cerebellar efferent system, in addition to motor neurons. In patients with pTDP-43-ir NCI, patients with NCI in the hippocampal dentate granule neurons (DG) showed a neuronal loss in the cerebral cortex, and patients without NCI in DG showed a preserved cerebral cortex. By contrast, in patients with FUS-ir NCI, patients with NCI in DG showed a preserved cerebral cortex and patients without NCI in DG showed marked cerebral degeneration. The cerebral cortex of patients with SOD1-ir NCI was preserved. Together, these findings suggest that lesions of the cerebrum are probably not necessary for progression to Stage V. In conclusion, patients with ALS that had progressed to result in communication Stage V showed rapidly-progressed symptoms, and their common lesions could cause the manifestations of communication Stage V.

Keywords: Amyotrophic lateral sclerosis, Communication Stage V, Totally locked-in state, Pallido-nigro-luysian system, Brainstem reticular formation, Cerebral cortical degeneration

Introduction

Patients with amyotrophic lateral sclerosis (ALS) dependent on tracheostomy with invasive ventilation (TIV) use mostly nonverbal communication and find it difficult to communicate as their muscle weakness progresses. In a previous study [9], we proposed a classification system for the communication abilities of patients with advanced ALS that consists of five stages: Stage I, communicates in sentences; Stage II, communicates with one-word answers only; Stage III, communicates with nonverbal yes/no responses only; Stage IV, occasionally cannot communicate due to uncertain yes/no responses; and Stage V, cannot communicate by any means. We also analyzed the relationship between clinical findings and the prognosis for communication disturbance [9, 19]. At present, communication Stage V is indicative of a "totally locked-in state" [7, 8]. Our previous analysis of 29 autopsies of patients with ALS who were dependent on TIV showed that seven patients who progressed to Stage V had begun to

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require TIV significantly earlier than patients who died in Stage IV or earlier, and the patients who progressed to Stage V frequently had a family history of ALS and gene mutation [9]. Further study showed that need for TIV, impaired oculomotor movement, and becoming totally quadriplegic within 24 months of ALS onset were predictors of severe communication impairment. Therefore, we recommended early detection of impaired communication and identification of the best methods of communication [19]. The first neuropathological reports of two patients with ALS who progressed to Stage V [7] showed severe multisystem degeneration. By contrast, Oyanagi et al. [22] reported marked preservation of the visual and olfactory pathways in patients with ALS who progressed to Stage V. There are reports of patients with lesions in their primary motor cortex, but a preserved cerebral cortex [10, 14, 16, 24]. In contrast, patients with marked cerebral atrophy due to degeneration of the cerebral cortex and white matter have also been reported [11, 17, 20]. Neuroradiologically, a progressive cerebral atrophy has been shown in siblings with ALS who carried a mutation in the gene for optineurin (*OPTN*) [28]. However, the distribution and characteristics of the cerebral lesions are unclear. To date, clinicopathology of patients with ALS who progressed to communication Stage V has been reported only in patient reports [7, 10, 11, 14, 16, 17, 20, 24, 26, 27]. Therefore, in the present study we performed a comprehensive analysis of the clinicopathological features and immunohistochemical characterization of patients with ALS who had progressed to communication Stage V.

Materials and methods

Patients

By examining medical records, we enrolled 11 (3.4 % of studied patients with ALS neuropathology) Japanese patients with ALS who had progressed to Stage V (Table 1), from among 320 patients with ALS neuropathologically confirmed at autopsy at the Tokyo Metropolitan Neurological Hospital between 1980 and 2012 (150 patients), and in the Department of Pathology Brain Research Institute, Niigata University between 1963 and 2012 (170 patients). No patients had clinical manifestations of either cognitive or behavioral impairment before progressing to Stage V. In addition, no patients showed clinical evidence of anoxia, such as suffocation, artificial ventilator accident, or blood pressure decrease to <80 mmHg with shock, during their clinical course. Several patients were reported elsewhere as having ALS that had progressed to Stage V [7, 10, 16, 17, 20, 24]; however, some of the older reports did not provide immunohistochemical characterization of patient specimens. The clinicopathological features of patients 4 and 11 were previously reported as motor neuron disease [27] and we reevaluated these patients as having phosphorylated TAR DNA-binding protein 43 (pTDP-43)-immunoreactive (ir) neuronal cytoplasmic inclusions (NCI) and copper/zinc superoxide dismutase (SOD1)-ir NCI respectively. Therefore, we included them in the present study, and reevaluated the clinicopathological and immunohistochemical features of all 11 patients using the same criteria. Of the 11 patients, six (patients 1–6) had pTDP-43-ir NCI, two (patients 7, 8) had fused in sarcoma (FUS)-ir NCI, and three (patients 9–11) had SOD1-ir NCI (Table 1). This study was approved by the Ethical Review Boards of Tokyo Metropolitan Neurological Hospital and Niigata University.

Histopathology

Specimens from the brain and spinal cord were fixed with 20 % buffered formalin and embedded in paraffin wax. Loss of neurons or fibers, or both, and gliosis were assessed in various regions of the nervous system using 10 or 4 μm sections in hematoxylin and eosin and Klüver–Barrera stains. When necessary, Bodian and Holzer stains were additionally used.

For immunohistochemistry, 6 or 4 μm sections were prepared. Sections from the frontal lobe, temporal lobe, hippocampus, parietal lobe, occipital lobe, basal ganglia, thalamus, cerebellum, midbrain, pons, medulla oblongata, and spinal cord were immunostained for pTDP-43, using a rabbit polyclonal antibody against pTDP-43 (pS409/410; CosmoBio, Tokyo, Japan) at a dilution of 1:5000 or 1:8000, FUS, using a rabbit polyclonal antibody against FUS (Sigma-Aldrich St. Louis, MO, USA) at a dilution of 1:2000, SOD1, using a rabbit polyclonal antibody against SOD1 (Proteintech, Tokyo, Japan) at a dilution of 1:100. Required sections were immunostained for phosphorylated tau protein using the mouse monoclonal antibody AT8 (Innogenetics, Ghent, Belgium) at a dilution of 1:200, α-synuclein using a mouse monoclonal antibody against α-synuclein (Wako, Osaka, Japan) at a dilution of 1:8000 or 1:10,000, and ubiquitin using a rabbit polyclonal antibody against ubiquitin (Dako, Glostrup, Denmark) at a dilution of 1:800. Before antibody incubation, sections were treated by microwaving in citrate-buffered saline (pH 6.0, 15 min) to unmask antigens. Antibody binding was visualized using a labeled streptavidin–biotin immunoperoxidase method. The chromogen and counterstain were diaminobenzidine and hematoxylin, respectively. NCI in each patient was assessed by immunostaining with pTDP-43, FUS, or SOD1.

We evaluated the degree and extent of the neuronal loss, gliosis, and NCI semiquantitatively. Degeneration was assessed as the degree of neuronal loss and gliosis and was indicated as absent (–), slight (+) (as shown in Fig. 3i), mild (++) (as shown in Fig. 3e-g), or severe (+++) (as shown in Fig. 3a-d, h). The frequency of NCI, evaluated at 200X magnification, was indicated as follows: none (0), no NCI

Table 1 Clinical characteristics

Patients	Sex	Age at onset(years)	Disease duration(months)	Disease duration (months)	Time from onset to clinical event (months)				Time from Stage V to death(months)	Accumulated protein	Reference
					Need for tracheostomy invasive ventilation	Progression to total quadriplegia	Development of overt oculomotor limitation	Resulting in communication Stage V[a]			
1	F	62	104	LE	12	34	33	37	67	TDP-43	[20]
2	F	52	161	UE	12	25	14	76	85	TDP-43	
3	F	53	120	B	56	120	120	120	0	TDP-43	
4	M	73	78	B	5	na	11	29	49	TDP-43	[27] patient 2
5	M	60	117	UE	15	31	18	81	36	TDP-43	[7] case 1
6	M	64	57	UE	12	33	33	56	1	TDP-43	[7] case 2
7	F	13	312	LE	18	18	36	120	192	FUS	[17] patient 1
8	M	39	102	LE	24	66	86	102	0	FUS	[16]
9	F	38	106	LE	8	9	11	36	70	SOD1	[24]
10	M	57	128	B	21	45	52	84	44	SOD1	[10]
11	M	61	29	UE	5	6	19	28	1	SOD1	[27] patient 1
mean		52.0	119.5		17.0	38.7	39.4	69.9	47.8		

F female, *M* male, *na* not available, *TDP-43* TAR DNA-binding protein 43, *FUS* fused in sarcoma, *SOD1* copper/zinc superoxide dismutase 1, *LE* lower extremity, *UE* upper extremity, *B* bulbar

[a]Communication Stage V is indicative of a "totally locked-in state" [7, 8], in which all voluntary movements are lost and communication is impossible by any means

across the entire section; rare (1), an average of <2 NCI per 5 fields; occasional (2), an average of 2–10 NCI per 5 fields; and frequent (3), an average of >10 NCI per 5 fields. For the semiquantitative analysis, two neuropathologists (K.H. and Y.M.) observed the specimens, and their scores were almost coincident.

Results

Clinical characteristics

Except for patient 7 with FUS-ir NCI, all patients had adult onset ALS (Table 1). The time from ALS onset to need for TIV was less than 24 months in ten patients, and 56 months in patient 3 with pTDP-43-ir NCI. All the patients progressed to have total quadriplegia and developed overt oculomotor limitation after using TIV. Development of oculomotor limitation started before or after they developed total quadriplegia, and the patients developed vertical gaze palsy followed by horizontal gaze palsy. Subsequently the patients showed slow eye movement and ultimately became ophthalmoplegic. All the patients used only eye movement for communication just before progressing to Stage V.

Macroscopic findings

Brain weight ranged from 610 to 1395 g (mean 1032.3 g) (Table 2) before formalin fixation. Four patients (patients 1, 2 (Fig. 1b), and 3 with pTDP-43-ir NCI, and 7 with FUS-ir NCI (Fig. 1a)) showed severe cerebral atrophy, which was a brain weight of <1000 g. They showed marked atrophy of both the pons and midbrain. The brain weight of seven patients (patients 4 (Fig. 1c), 5 and 6 with pTDP-43-ir NCI, 8 with FUS-ir NCI, and 9–11 with SOD1-ir NCI) was >1000 g, and their pons and midbrain showed mild to moderate atrophy. All patients showed severe atrophy of the spinal cord and the medulla oblongata. The optic nerve (Fig. 1) and the lateral geniculate body of all the patients appeared preserved.

Microscopic findings

Table 2 shows the neuropathological findings of the patients.

Lesions of the motor system

All the patients showed a severe loss of Betz cells. The other neurons in the motor cortex were mildly decreased with a few NCI in the patients with mild cerebral atrophy (patients 4–6, and 8–11). By contrast, the patients with severe cerebral atrophy (patients 1–3, and 7) showed severe neuronal loss with gliosis and many NCI in layer 2 and the deeper layer of the motor cortex. Basophilic inclusions were frequently observed in patient 7 and rarely observed in patient 8. The pyramidal tract showed severe fiber loss, although a few small fibers remained in the medullary pyramid and both the lateral and anterior corticospinal tract in the spinal cord. The anterior horn of the spinal cord and the tegmentum of the brainstem including the hypoglossal nucleus showed severe atrophy (Fig. 2) and severe neuronal loss with a few NCI in the atrophied neurons. The facial and the trochlear nuclei, which were confirmed in patients 3 and 11 showed severe neuronal loss with gliosis. The oculomotor nucleus showed a difference in the degree of degeneration. We were not able to evaluate the exact occurrence of NCI in the lesions, because of severe neuronal loss.

Lesions of the extrapyramidal motor system and nonmotor system

Neuronal and fiber loss with gliosis were observed in the substantia nigra (Fig. 3a–c), globus pallidus (Fig. 3d–f), subthalamic nucleus, brainstem reticular formation, cerebellar dentate nucleus, superior cerebellar peduncle, red nucleus, Clarke's nucleus, and posterior spinocerebellar tract in all the patients. In particular, the degeneration of the substantia nigra (Fig. 3a–c), globus pallidus (Fig. 3d–f), subthalamic nucleus, and brainstem reticular formation (Fig. 2) was moderate or severe in all patients regardless of the time for progression from Stage V to death, or the type of accumulated proteins. By contrast, the cerebellar efferent system, which consists of the cerebellar dentate nucleus, superior cerebellar peduncle, and red nucleus, showed mild to moderate degeneration in patients 3, 5, and 6 with pTDP-43-ir NCI, in patient 8 with FUS-ir NCI, and in patient 11 with SOD1-ir NCI. The time for progression from Stage V to death was shorter in these five patients (0–36 months) than in the other six patients (44–192 months). The cerebellar afferent system, which consists of the pontine nuclei and middle cerebellar peduncle, was relatively preserved. However, in patient 8, who had a p.K510M mutation in the gene for FUS (*FUS*) [16], showed severe degeneration in the cerebellar afferent pathway. Patient 7, who had a p.P525L mutation in *FUS* and severe frontal lobe atrophy [17], showed severe degeneration in the caudate nucleus and putamen (Fig. 3h) and moderate degeneration in the globus pallidus (Fig. 3e). The caudate nucleus and putamen (Fig. 3g, i) were relatively better preserved than the globus pallidus (Fig. 3d, f), except for in patient 7. The inferior olivary nucleus in patients 2 and 10 showed vacuolar degeneration in neurons and an increase of gemistocytic astrocytes, indicating inferior olivary hypertrophy [5, 10]. For pTDP-43, FUS, and SOD1, no patients exhibited accumulation of multiple different proteins. AT8 immunostaining of neurofibrillary tangles revealed a Braak stage of ≤ II [1]. No patients had α-synuclein-ir structures. In addition, among patients with pTDP-43-ir NCI, no patients showed displayed ubiquitin-ir NCI in cerebellar granule cells or hippocampal CA4 subfield neurons.

Table 2 Neuropathological findings

Patients	1		2		3		4		5		6		7		8		9		10		11	
Brain weight (g)	610		783		930		1395		1050		1190		715		1233		1019		1170		1260	
Accumulated protein	TDP-43		TDP-43		TDP-43		TDP-43		TDP-43		TDP-43		FUS		FUS		SOD1		SOD1		SOD1	
Gene mutation	n		n		n		n		n		n		p.P525L		p.K510M		p.V118L		n		p.C146R	
NCI in the dentate granule neuron	+		+		+		+		-		-		-		+		-		-		-	
	DEG	NCI	DEG	NCI	DEG	NCI	DEG	NCI	DEG	NCI	DEG	NCI	DEG	NCI	DEG	NCI	DEG	NCI	DEG	NCI	DEG	NCI
Cerebrum																						
Frontal	n	1	+	2	+	1	+	1	+	1	+	1	++	2	-	0	-	0	-	0	-	0
Temporal	n	1	+	2	++	2	+	1	-	0	+	0	+	1	-	0	-	1	-	0	-	0
Hippocampal subiculum	n	2	++	2	++	3	+	2	-	0	-	0	-	0	-	0	-	0	-	0	-	0
Occipital	n	1	-	1	-	1	+	1	-	0	-	0	-	2	-	1	-	0	-	0	-	0
Motor neurons																						
Primary motor cortex	n	2	+++	3	++	2	++	2	+	1	++	2	++	2	+	1	+	1	+	0	+	2
Corticospinal tract	+++	n	+++	n	+++	n	+++	n	+++	n	+++	n	+++	n	+++	n	+++	n	+++	n	+++	n
Hypoglossal nucleus	+++	0	+++	0	+++	0	+++	0	+++	0	+++	0	+++	0	+++	0	+++	0	+++	0	+++	1
Anterior horn of the spinal cord	+++	1	+++	0	+++	1	+++	1	+++	0	+++	1	+++	1	+++	1	+++	1	+++	0	+++	1
Eye movement system																						
Oculomotor nucleus	+++	2	+++	1	+	1	+++	1	+	1	+	1	+++	0	++	1	+++	0	+++	0	++	2
Medial longitudinal fasciculus	++	n	+++	n	+	n	++	n	++	n	++	n	+++	n	++	n	+++	n	+++	n	+	n
Superior colliculus	+++	2	+++	1	++	1	+	1	+	n	+	n	+++	0	+	2	++	2	++	0	+	1
Brainstem reticular formation																						
Pontine reticular formation	+++	2	+++	2	++	1	++	1	++	2	++	3	+++	0	++	3	+++	2	+++	0	++	3
Medullary reticular formation	+++	2	+++	2	+++	1	+++	2	+++	2	+++	3	+++	0	+++	2	+++	2	+++	0	++	3
Spinal cord																						
Anterolateral funiculus	+++	n	+++	n	+++	n	+++	n	+++	n	+++	n	+++	n	+++	n	+++	n	+++	n	+++	n
Spinocerebellar tract	++	n	+++	n	++	n	+++	n	+++	n	+++	n	+++	n	+++	n	+++	n	+++	n	++	n
Intermediolateral nucleus	++	1	++	1	+	0	++	1	+	1	+	1	++	2	++	1	+++	0	+++	0	+	1
Clarke's nucleus	++	1	+++	1	+	0	++	1	++	1	++	1	++	0	++	1	+++	0	+++	0	++	1
Posterior column	-	n	++	n	+	n	+	n	++	n	+	n	+	n	++	n	+++	n	+++	n	++	n
Substantia nigra and basal ganglia																						
Substantia nigra	+++	2	+++	1	++	2	++	1	++	1	++	1	+++	0	++	3	+++	0	+++	0	++	2
Globus pallidus	n	n	+++	1	++	2	++	3	++	2	++	2	++	0	++	3	++	2	++	0	++	2
Subthalamus nucleus	n	n	+++	3	++	2	++	n	n	n	++	n	+++	1	++	n	++	2	++	0	++	3
Caudate nucleus	n	n	+++	1	+	2	+	1	+	1	-	1	+++	0	+	2	-	1	+	0	+	1
Putamen	n	n	++	1	+	3	+	1	+	1	-	1	+++	1	-	2	+	2	+	0	+	1
Cerebellum and related area																						
Purkinje cells	++	0	+	0	-	0	-	0	-	1	-	0	++	0	-	0	-	0	+	0	-	0
Cerebellar dentate nucleus	++	1	+++	2	+	0	++	1	+	2	+	2	+++	1	+	2	+++	1	+++	0	+	1
Superior cerebellar peduncle	++	n	+++	n	+	n	+	n	+	n	+	n	+++	n	+	n	+++	n	++	n	+	n
Red nucleus	++	1	+++	0	+	0	++	1	++	1	++	1	+++	0	+	2	+++	1	+++	0	+	1
Central tegmental tract	+++	n	+++	n	+	n	+	n	+	n	+	n	+++	n	+	n	+++	n	+++	n	+	n
Inferior olivary nucleus	+	3	++	3	+	1	+	2	+	2	+	3	++	2	+	3	++	1	++	0	+	1
Pontine nucleus	-	2	+	3	-	2	+	1	-	1	-	2	++	1	+++	3	-	2	+	0	+	2
Middle cerebellar peduncle	-	n	-	n	-	n	+	n	-	n	-	n	++	n	+++	n	-	n	-	n	-	n
Cerebellar white matter	n	n	+++	n	++	n	+	n	+	n	+	n	+++	n	++	n	+	n	+	n	-	n
Senrory system																						
Medial lemniscus	+	n	++	n	-	n	+	n	+	n	+	n	++	n	+	n	+	n	++	n	-	n
Primary sensory cortex	n	1	+	1	+	2	+	1	+	1	+	2	+	0	+	1	+	0	+	0	+	1
VPM of the thalamus	n	n	++	2	+	1	++	2	+	n	-	2	++	1	+	2	++	2	+	0	+	2
Visual system																						
Optic tract	-	n	-	n	-	n	-	n	-	n	-	n	-	n	-	n	-	n	-	n	-	n
Lateral geniculate bony	n	n	n	n	-	0	+	1	-	n	-	n	-	0	-	n	-	n	-	0	-	n
Optic radiation	n	n	-	n	-	n	-	n	-	n	-	n	-	n	-	n	-	n	-	n	-	n
Striate cortex	n	1	-	1	-	1	+	1	-	0	-	0	-	2	-	1	-	0	-	0	-	0
Auditory system																						
Superior olivary nucleus	+++	1	++	1	-	1	+	1	+++	1	-	0	n	n	+++	n	+	1	+++	0	+	1
Lateral lemniscus	+	n	++	n	n	n	+	n	+++	n	+	n	n	n	+++	n	++	n	+++	n	-	n
Nucleus basalis of Meynert	n	n	-	n	n	n	-	1	-	0	-	1	-	-	n	n	-	0	-	0	-	1

DEG degeneration assessed degree of neuronal loss and gliosis on the hematoxylin and eosin, and Klüver–Barrera-stained sections
The degeneration was indicated as absent (–); slight (+); mild (++); or severe (+++)
NCI neuronal cytoplasmic inclusions, The NCI was indicated as none (0); rare (1); occasional (2); or frequent (3), yellow means that we were not able to evaluate the exact occurrence of NCI in the lesions, because of moderate to severe neuronal loss
n not evaluated (or not examined), *VPM* ventral posterior medial nucleus

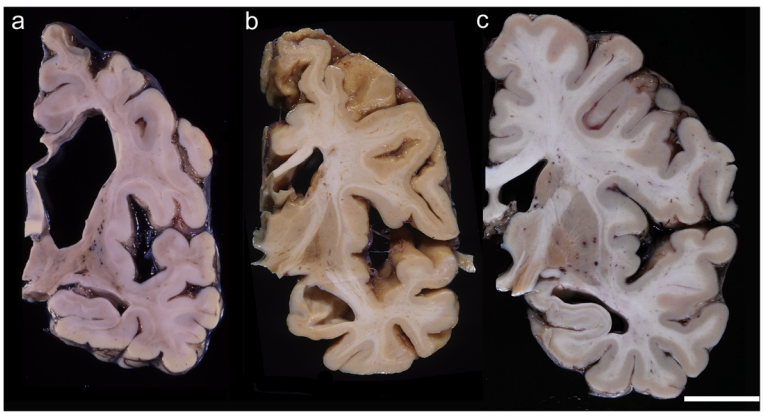

Fig. 1 Coronal sections of the cerebrum at the subthalamic nucleus. **a** Patient 7 with fused in sarcoma (FUS)-immunoreactive (ir) neuronal cytoplasmic inclusions (NCI) showed enlargement of the anterior horn of the lateral ventricle, and atrophy of the caudate nucleus, putamen, and frontal lobe, particularly in the frontal white matter along with a thin corpus callosum. However, the hippocampus and temporal lobe were preserved. **b** Patient 2 with phosphorylated TAR DNA-binding protein 43 (pTDP-43)-ir NCI showed temporal predominately frontotemporal atrophy. **c** Patient 4 with pTDP-43-ir NCI showed no white matter atrophy nor enlargement of the lateral ventricle. The globus pallidus and subthalamic nucleus were brownish with mild to severe atrophy, while the caudate nucleus and the putamen were relatively preserved in all patients except for patient 7 (**a**), and the optic nerve was preserved in all patients. (Bar = 2 cm)

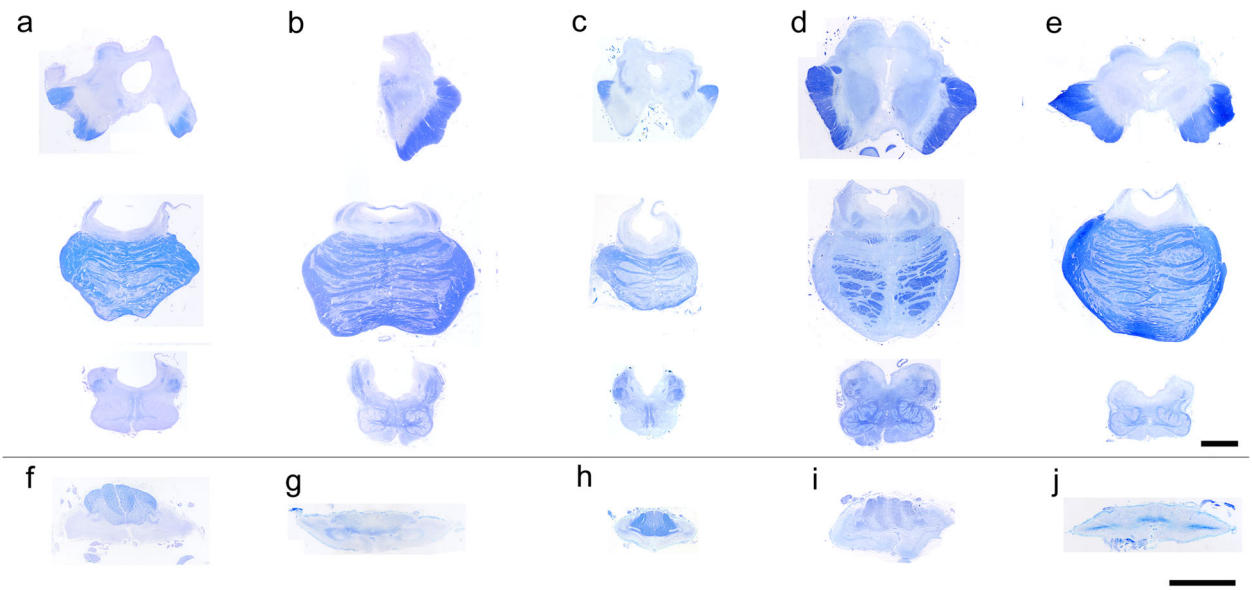

Fig. 2 Brainstem and seventh cervical cord at the same magnification respectively. All patients showed marked nerve fiber loss of the brainstem tegmentum including the brainstem reticular formation and both lateral and anterior funiculus in addition to pyramidal tract, and showed marked atrophy of the anterior horn of the spinal cord. The medulla oblongata and spinal cords showed severe atrophy and the fourth ventricle was markedly dilatated with severe degeneration of the hypoglossal nucleus in all patients. The midbrain and pons showed severe atrophy in patients 2 with phosphorylated TAR DNA-binding protein 43 (pTDP-43)-immunoreactive (ir) neuronal cytoplasmic inclusions (NCI) (**a**) and patient 7 with fused in sarcoma (FUS)-ir NCI (**c**) in whom brain weight was less than 1000 g, mild to moderate atrophy in patient 5 with pTDP-43-ir NCI (**b**), patient 8 with FUS-ir NCI (**d**), and patient 10 with copper/zinc superoxide dismutase (SOD1)-ir NCI (**e**) in whom brain weight was more than 1000 g. The superior cerebellar peduncle in patients 5 (**b**) and 8 (**d**) were degenerated mildly. Loss of transverse fibers of the pons and middle cerebellar peduncle were observed only in patient 8 (**d**). The posterior column of the spinal cord showed a marked loss of fibers in patients 5 (**g**) and 10 (**j**), but they were relatively preserved in patient 7 (**h**), and fiber loss of the middle root zone in patient 2 (**f**) and 8 (**i**). **a**, **f** Patient 2 with pTDP-43-ir NCI. **b**, **g** Patient 5 with pTDP-43-ir NCI. **c**, **h** Patient 7 with FUS-ir NCI. **d**, **i** Patient 8 with FUS-ir NCI. **e**, **j** Patient 10 with SOD1-ir NCI, (Bar = 5 mm, Klüver–Barrera staining)

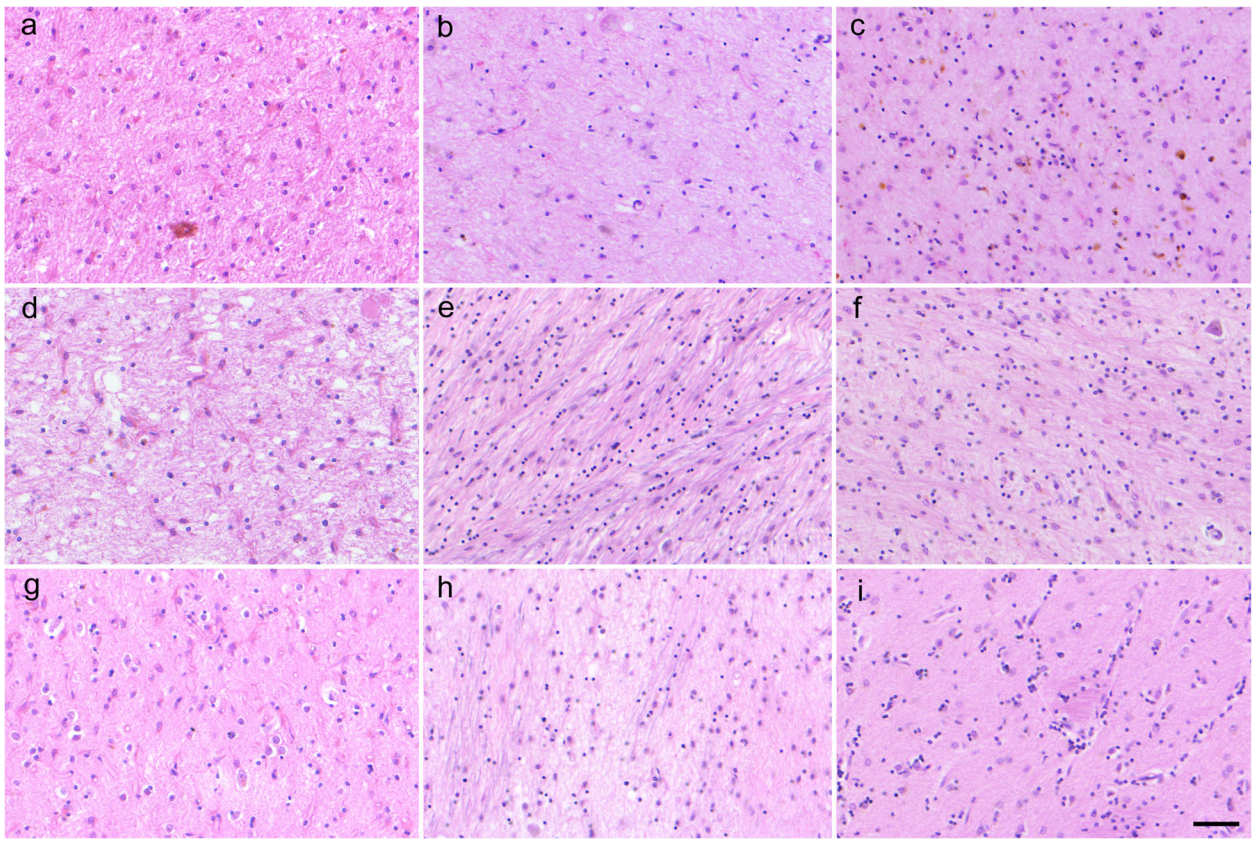

Fig. 3 Lesions of the nonmotor system. **a**–**c** Substantia nigra showing severe neuronal loss with gliosis. **d**–**f** Globus pallidus, **g**–**i** putamen. Only patient 7 showed more severe degeneration in the putamen (**h**) than in the globus pallidus (**e**). Other patients showed more severe degeneration in the globus pallidus (**d**, **f**) than in the putamen (**g**, **i**). **a**-**d**, **h** neuronal loss and gliosis were severe (+++). **e**-**g** neuronal loss and gliosis were mild (++). **i** neuronal loss and gliosis were slight (+). **a**, **d**, **g** Patient 2 with phosphorylated TAR DNA-binding protein 43-immunoreactive (ir) neuronal cytoplasmic inclusions (NCI). **b**, **e**, **h** Patient 7 with fused in sarcoma-ir NCI. **c**, **f**, **i** Patient 10 with copper/zinc superoxide dismutase-ir NCI, (Bar = 50 μm, hematoxylin and eosin staining)

Preserved areas

The optic tract and optic radiation were well preserved in all the patients (Fig. 4). The lateral geniculate body was examined in nine patients, of which eight showed comparative preservation. In the striate cortex, although there were a few NCI in six patients (patients 1–4 with pTDP-43-ir NCI, and patients 7 and 8 with FUS-ir NCI), mild neuronal loss and gliosis were observed only in patient 4. The nucleus basalis of Meynert was examined in eight patients, showing no neuronal loss although a few NCI were found in patients 4, 6 with pTDP-43-ir NCI and in patient 11 with SOD1-ir NCI.

Cerebral cortical findings by accumulated protein

Among six patients with pTDP-43-ir NCI (patients 1–6), four (patients 1–4) had NCI in the hippocampal dentate granule neurons and showed neuronal loss and gliosis with NCI in the hippocampal subiculum, and the frontal and temporal cortex (Fig. 5a–c). Patients 5 and 6, lacking NCI in the dentate granule neurons in the hippocampus, demonstrated the occurrence of NCI, limited to the frontal cortex, in the absence of neuronal loss (Fig. 5d–f). The pTDP-43-ir pathological pattern of all six patients was consistent with type B [12].

One (patient 7) with FUS-ir NCI showed frontal and temporal cortical degeneration with compacted occurrence of smaller NCI in the absence of NCI in the hippocampal dentate granule neurons (Fig. 5g–i). The other (patient 8) had noncompact NCI in the hippocampal dentate granule neurons, and their cerebral cortex was preserved with rare NCI (Fig. 5j–l).

The cerebral cortex, with the exception of the motor cortex, of all three patients with SOD1-ir NCI was preserved. A few NCI were scattered in the temporal cortex only in patient 9.

Discussion

As noted in our previous reports [9, 19], patients with ALS that progressed to Stage V showed rapid clinical

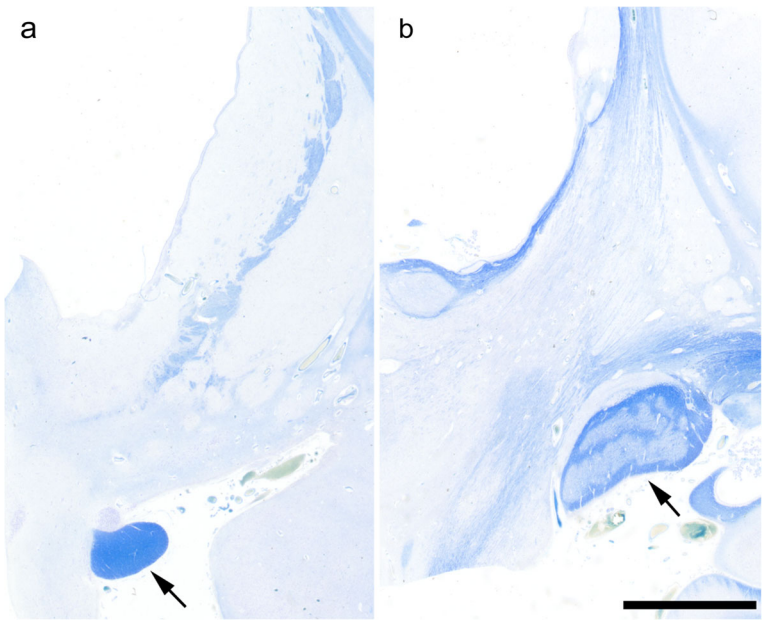

Fig. 4 Preserved areas. **a** Preserved optic tract (arrow) and severe degeneration of the other regions. **b** Preserved lateral geniculate body (arrow) and severe degeneration of other regions. (Patient 7, Bar = 5 mm, Klüver–Barrera staining)

deterioration, and tended to need TIV within 24 months of ALS onset. The onset of lesions varied, and the onset lesions and the time from onset to resulting in Stage V did not appear to be related. Subsequently, their condition progressed to Stage V due to complete ophthalmoplegia in addition to total quadriplegia. This clinical course is similar to that of the previously reported patients who progressed to Stage V (Table 3) [11, 14, 26, 28]. However, a few patients required TIV over 24 months after the onset, such as our patient 3 with pTDP-43-ir NCI, and three patients reported in the existing literature, showing pTDP-43-ir NCI [11], basophilic inclusions [14], and a mutation in *OPTN* [28], respectively (Tables 1 and 3). These patients showed that patients who required TIV after more than 24 months from disease onset could not necessarily avoid progression to Stage V.

Neuropathologically, regardless of the type of accumulated proteins, all the patients shared the feature of severe degeneration in the substantia nigra, globus pallidus, subthalamic nucleus, brainstem reticular formation, and mild to severe degeneration of the cerebellar efferent system, in addition to the severe degeneration in both upper and lower motor neurons. It is likely that the constellation of such lesions is common in patients with ALS who progress to Stage V, because the patients reported in the literature who had progressed to Stage V also demonstrated a similar constellation (Table 3) [11, 14, 26]. In addition, Miki et al. [13] reported a patient with ALS who required TIV and did not progress to Stage V, and discussed this with regard to pallido–nigro–luysian degeneration. They concluded that the pallido–nigro–luysian degeneration may be involved in the ALS disease process [13]. Another patient with ALS who required TIV and did not progress to Stage V also showed pallido–nigro–luysian degeneration [23]. These two patients who did not progress to Stage V showed no apparent degeneration in the brainstem reticular formation or cerebellar efferent system, although they did show pallido-nigro-luysian degeneration similar to the patients who progressed to Stage V [13, 23]. Therefore, it is likely that the degeneration of the brainstem reticular formation and the cerebellar efferent system in addition to degeneration of the pallido–nigro–luysian system is necessary for progression to Stage V.

Relatively mild degeneration of the cerebellar efferent system was seen in patients who died shortly after progressing to Stage V (patients 3, 5, 6, 8, 11) and has been reported in one patient [26]. The time for progression from Stage V to death may alter the degree of degeneration in the cerebellar efferent system.

Neuroanatomically, the common lesions of patients with ALS who progressed to Stage V appeared to be related to each other through connections with the motor-related area (Fig. 6) [4]. The degeneration of motor neurons, which are the basic lesions in ALS, is so severe in patients who progress to Stage V that the degeneration of associated areas appeared to occur secondarily due to degeneration of motor neurons. We could not evaluate the precise relationship between neuronal loss and occurrence of NCI because of the severe neuronal loss. Brettschneider et al. described a pTDP-43 staging

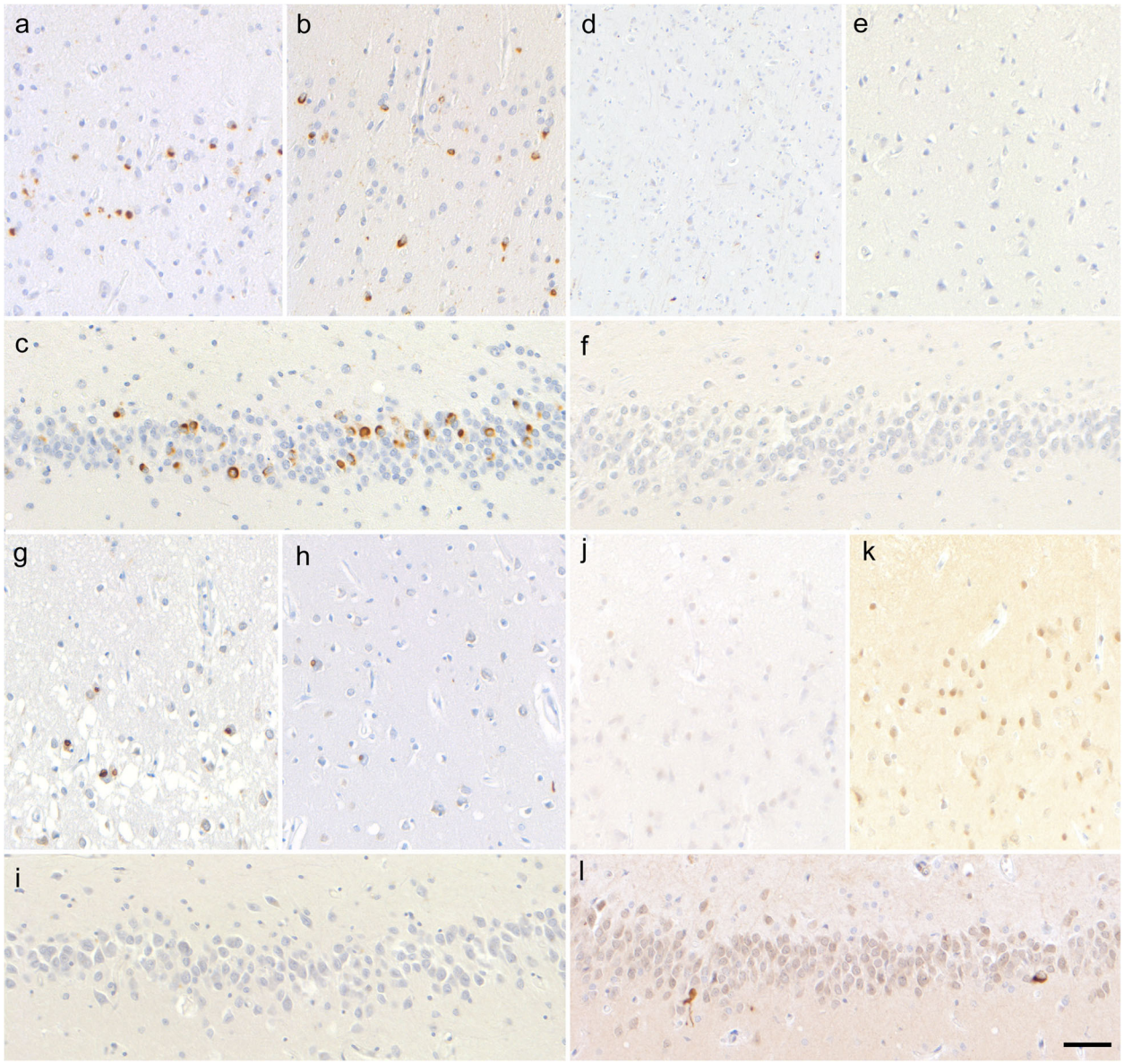

Fig. 5 Immunohistochemical features of cerebral lesions. **a**–**c** Patient 2 with phosphorylated TAR DNA-binding protein 43 (pTDP-43)-immunoreactive (ir) neuronal cytoplasmic inclusions (NCI) in the hippocampal dentate granule neurons (**c**) had occasional NCI in the frontal (**a**) and temporal (**b**) cortex. **d**–**f** Patient 6 without pTDP-43-ir NCI in the hippocampal dentate granule neurons (**f**) had rare NCIs in the frontal cortex (**d**) and no NCIs in the temporal cortex (**e**). **g**–**i** Patient 7 with occasional fused in sarcoma (FUS)-ir NCI in the frontal cortex (**g**) and rare NCI in the temporal cortex (**h**) had no NCI in the hippocampal dentate granule neurons. **j**–**l** Patient 8 without FUS-ir NCI in the frontal (**j**) and temporal cortex (**k**) had rare NCI in the hippocampal dentate granule neurons (**l**). Bar = 50 μm

algorithm, and presented evidence for a possible sequential dissemination of pTDP-43 pathology [2, 3]. However, applying the stages of pTDP-43 to the present patients with pTDP-43-ir NCI, the inferior olivary nucleus, which was mentioned as an early lesion [2, 3], showed relatively mild neuronal loss even in communication Stage V, and the subthalamic nucleus, which was mentioned as a lesion having no NCI until late stages [2, 3], showed moderate to severe degeneration in communication Stage V. At least in the patients with pTDP-43-ir NCI, we speculate that the ease of occurrence of NCI and the speed of degeneration in the patients with ALS may differ depending on each lesion, as follows: (1) Lesions that showed a matched degree of appearance of NCI and neuronal loss, such as in the motor neurons, globus pallidus, and substantia nigra, (2) lesions that had NCI from an early disease phase without rapid neuronal loss, such as in the inferior olivary

Table 3 Clinicopathological characteristics of previously reported patients in communication Stage V

Sex	Age at onset (years)	Disease duration (months)	Time from onset to clinical event, months					
			Need for tracheostomy invasive ventilation	Progression to total quadriplegia	Development of overt oculomotor limitation	resulting in communication Stage V	Communication Stage V to death(months)	Accumulated protein
M	38	60	11	20	20	32	28	ubiquitin
M	38	156	60	132	96	144	12	ubiquitin[a]
M	63	144	36	na	36	na	na	TDP-43
F	33	288	24	na	na	156	84	a mutation in *OPTN*
M	35	240	36 ≥	na	na	36	204	a mutation in *OPTN*
M	44	27	6	15	15	26	1	na

Table 3 Clinicopathological characteristics of previously reported patients in communication Stage V *(Continued)*

Sex	Age at onset (years)	Neuropathology Brain weight	Upper and lower motor neurons	Substantia nigra	Globus pallidus	Subthalamic nucleus	Brainstem reticular formation	Cerebellar efferent system	Reference
M	38	1300	+++	+++	+++	na	+++	++	[14] Patient 1
M	38	1230	+++	+++	na	na	++	+++	[14] Patient 2
M	63	830	+++	+++	+	na	+++	+	[11]
F	33		N/A	N/A	N/A	N/A	N/A	N/A	[28] Case 1[b]
M	35		N/A	N/A	N/A	N/A	N/A	N/A	[28] Case 2[b]
M	44	1320	+++	+	+	+	+++	+	[26]

M male, *F* female, *na* not available, *N/A* not applicable, *OPTN* the optineurin gene
[a]ubiquitin-immunoreactive basophilic inclusions
[b]Clinical report

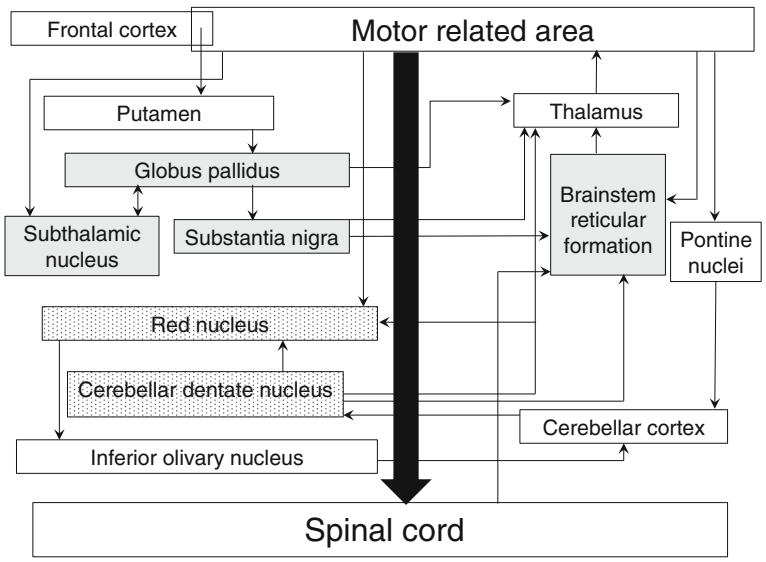

Fig. 6 The connections of motor neuron-related area. The common lesion of the ALS patients who progressed to Stage V is emphasized. All the common lesions related to the motor neurons and pyramidal tract. Shaded areas: lesions that showed moderate or severe degeneration in all the patients regardless of the time for progression to Stage V to death or type of accumulated protein. Stippled areas: lesions that showed a different degree of degeneration depending on the time from progression to Stage V to death

nucleus, (3) lesions although having rare NCI in early disease phases, subsequently showed severe neuronal loss, such as in the subthalamic nucleus, and (4) lesions that had rare NCI without obvious neuronal loss, such as in the lateral geniculate body and striate cortex in the visual pathway, which were preserved from lesions in patients who progressed to Stage V [22].

In this study, patient 2 with pTDP-43-ir NCI and patient 10 with SOD1-ir NCI [10] had inferior olivary hypertrophy. It is noteworthy that inferior olivary hypertrophy can occurs in ALS, regardless of accumulated protein, likely to progressive supranuclear palsy [6], Creutzfeldt–Jakob disease [15], or cerebrovascular disease [5].

A distinct pattern of protein accumulation was found in the cerebral cortex. First, in patients with pTDP-43-ir NCI (Table 2, Fig. 5a–f), patients with no NCI in the hippocampal dentate granule neurons (patients 5, 6) showed limited degeneration in the frontal cortex, and had relatively preserved brain weight. By contrast, patients with NCI in the hippocampal dentate granule neurons (patients 1–4) showed marked degeneration in the temporal cortex and hippocampal subiculum; and NCI were observed in the frontal, temporal, and occipital cortex. The characteristics of the cerebral cortical features in the present patients could be classified according to the presence of NCI in the hippocampal dentate granule neurons as described by Nishihira et al. [21]. Moreover, we identified that the patient with a Nishihira's Type 1 distribution pattern also progressed to Stage V. Interestingly, the relationship between NCI in the hippocampal dentate granule neurons and cerebral cortical degeneration showed a contrasting trend in patients with pTDP-43-ir NCI and FUS-ir NCI in this study as follows: patient 7 [17], who lacked NCI in the hippocampal dentate granule neurons showed severe atrophy of the cerebrum, whereas patient 8 [16], who had NCI, showed no atrophy of the cerebrum (Table 2, Fig. 5g–l). Second, the cerebral cortex of all 3 patients with SOD1-ir NCI was preserved with rare NCI. Patients with a mutation in *SOD1* were reported as having frontotemporal dementia (FTD) and decline of cognitive function was rare [25, 29]. Only one patient with a mutation in *SOD1* showing frontotemporal lobar degeneration (FTLD) underwent a detailed clinicopathological analysis [18]. Taking these findings together, it is likely that the cerebrum of patients with SOD1-ir NCI may be preserved even in the patients who progressed to Stage V. That there were three patients who showed a preserved cerebrum (patients 9–11), and that cerebral lesions shared by all patients and severe neuronal loss were missing in the present patients, suggest that cerebral cortical involvement was not necessary to progress to Stage V.

It is important to differentiate the present patients from patients showing FTLD. There is a limitation in that the decline of cognitive function cannot be assessed in the patients who progressed to Stage V. However, none of the patients had any clinical manifestation of cognitive or behavioral impairment while they could communicate, so they were not diagnosed as having FTD [25]. Furthermore, it was revealed that the patients who progressed to Stage V did not always show pathological characteristics of FTLD.

Conclusion

In the present study, we evaluated only a small number of patients because the patients who progressed to Stage V were limited to 3.4 % of all patients with ALS who underwent autopsy. Nevertheless, we identified that the patients who progressed to Stage V, including the patients reported previously, had common lesions in the pallido–nigro–luysian system, brainstem reticular formation, cerebellar dentate nucleus, superior cerebellar peduncle, and red nucleus, besides motor neurons. Moreover, we clarified specific characteristics in the relationship between accumulated protein and the cerebral cortical lesions. Thus, there may be common lesions that can identify patients with ALS who progress to Stage V. We consider that attention to the occurrence of symptoms of the extrapyramidal motor system and nonmotor system, and a radiological assessment of common lesions are important for predicting communication disability, especially in the patients who progressed rapidly from disease onset to TIV.

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Authors' contributions

KH and YM designed the study and performed semiquantitative analysis. KH, YM, RT, KW, NA, KO, MH, HT, and AK performed the pathological observations and evaluations. KH, ST, MN, OO, and EI performed clinical evaluations. KW, NA, OK, MH, HT, AK, and EI supervised the whole process of the study. KH and YM wrote the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests. The authors alone are responsible for the content and writing of the paper.

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