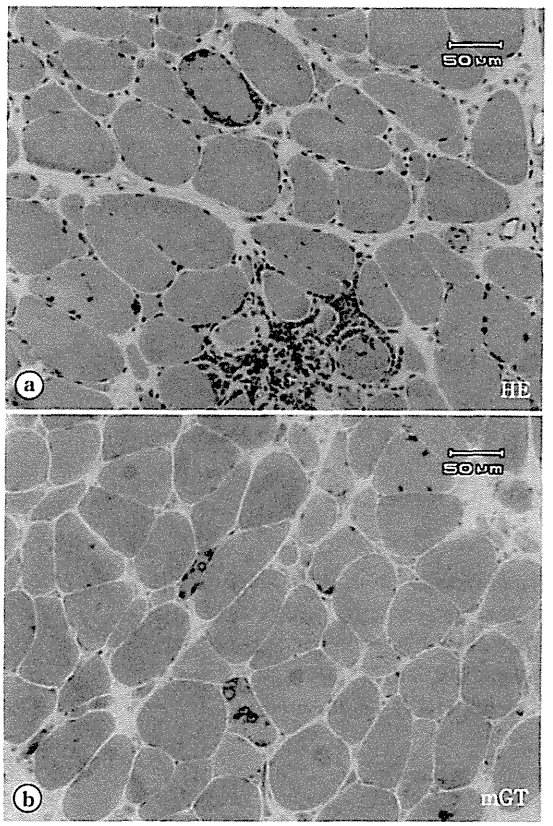

図4 sIBMの筋生検病理像

a. ヘマトキシリン・エオジン(HE)染色. b. ゴモリトリクローム変法(mGT).
単核球の炎症細胞が, 非壊死性筋線維を取り囲んでいる. HE染色で紫色, mGT染色で赤紫色顆粒にて縁取られたrimmed-vacuole陽性の筋線維の周辺には炎症細胞浸潤は認めない.

ciency virus)[17]やHTLV-1(human T-lymphotrophic virus-Type 1)[18]感染患者にてIBMを併発することがあり, その際にendomysiumに浸潤して非壊死線維を取り囲むCD8リンパ球のT細胞レセプターのサブタイプも限られている. 以上のことから, いまだ抗原は見つかっていないが, sIBMにみられるT細胞の反応は抗原特異的と考えられる[19].

8 βアミロイド仮説

sIBM患者の生検筋の免疫染色では, アミロイドβタンパク(Aβ)やアミロイドβ前駆タンパク(AβPP), リン酸化タウ, プリオンタンパク, アポリポタンパクE, α-1アンチキモトリプシンなどが, RV内に存在することから, Askanasらがアミロイドβ仮説を提唱した[20]. sIBM患者骨格筋では, AβPPの転写が促進されており[21], さらにβ-cite APP cleaving enzyme 1(BACE1)やγセクレターゼといったAβPPからAβを産生させる酵素の発現も増加している[22][23]. Aβがどのようにして骨格筋を障害するかははっきりしないが, AβPPを筋特異的に発現させたマウスでは, 筋変性や封入体が形成されたとの報告や[24], 炎症細胞からの各種サイトカインが骨格筋内のAβPPの産生を増強すると報告されている[25]. dysferlinは, 筋細胞膜の修復因子であり, LGMD2Bでは免疫染色にて筋細胞膜での発現

が低下している．sIBMの生検筋を使った免疫染色にて，dysferlinとアミロイドβ42の局在が類似しており，sIBMでの両者の関係が注目されている[26]．

9 筋核異常

TAR DNA-binding protein of 43 kDa(TDP-43)は，多くの組織や細胞で恒常的に発現しているRNA結合タンパクである．細胞内では主として核内に存在するが，核外や核内移行シグナルを有しているため，核と細胞質を行き来している．近年，TDP-43はALSや前頭側頭葉変性症に異常蓄積していることが判明した．sIBMでは筋核内のTDP-43が減少した筋線維では，TDP-43が筋細胞質内に集積している[27,28]．sIBMでは，種々のタンパクが細胞質内に沈着するが，TDP-43は，最も高頻度に沈着し，筋線維内の23％に及ぶとの報告もある．筋核からTDP-43が消失すると，核の形態変化が出現し，アポトーシスに陥る[29]．TDP-43の筋細胞質内分布がsIBMの病態に関与すると考えられている[27]．しかし，細胞質内へのTDP-43の沈着は，sIBMに特異的ではなく[30,31]，筋細胞質への沈着は少数であるという報告もあり検討を要する[32,33]．

10 ユビキチン-プロテアソームシステム

ユビキチンは，まず標的タンパクに結合し，その不要タンパクをプロテアソームが分解する．ユビキチンは，ATPのエネルギーを使用して，活性化される．ユビキチンリガーゼE3は，この活性化したユビキチンと標的タンパクとの仲介作用を有している．sIBMでは，ユビキチンリガーゼE3の一つであるring finger protein 5(RNF5)の発現が増加し，過剰発現させたマウスでは，筋萎縮・筋線維の壊死・再生と封入体形成が確認されたと報告されている[34]．一方，sIBMではプロテアソームの機能低下も報告されており[35,36]，ユビキチン-プロテアソーム系の異常が推定されている．

11 オートファジー

オートファジーは，タンパク質分解システムの一つで，細胞質にあるタンパク質やオルガネラを非選択的にオートファゴソームといわれる脂質膜で取り囲みライソゾームで分解することで細胞の恒常性維持を行っている．当初，オートファジーは，プロテアソーム系とは異なり，対象とする基質タンパクに選択性がないものと考えられていた．しかし，プロテアソームで分解できなかったユビキチン化したタンパクがオートファジーにて分解されることがわかり，さらにp62タンパク(別名：sequestosome 1(SQSTM1))がオートファジーの基質選択性を与える主要なアダプタータンパクであることが明らかになった[37]．つまり，タンパク質を取り囲むオートファゴソームは，LC3タンパクが，脂質膜とともに隔離膜と融合し，伸長して形成される．このときオートファゴソームと基質タンパクをつなぐのがp62である．このp62やLC3はsIBMでは，高度に発現している[38]．選択的オートファジーのその他のアダプタータンパクにはneighbor of BRCA 1 gene 1(NBR1)やオプチニューリンがあり，いずれもsIBMでは発現が確認されている[39,40]．これらのタンパク増加が，sIBMでの筋障害の原因であるのか，単にオートファジーが亢進しているのかは不明であるが，LC3とAβPP/AβがsIBMの生検筋内の空胞内に共存していることから，オートファジーを介し，ライソゾームを分解する際のターゲットにAβが関与している可能性がある．さらに，これらの筋線維は，MHC ClassⅠやⅡを発現しており，その前まわりをCD4または，CD8陽性リンパ球が取り囲んでいる．以上のことは，筋変性としてのAβ，自己貪食のLC3が炎症機転と関連している可能性を示唆している[41]．

一方sIBMでは，骨格筋内のライソゾーム機能が抑制され，オートファジー機能が低下しているとの報告もあり議論を要す[42]．さらに，培養筋を用いた検討で，IFN-1β，Il-6，TNF-αといったサイトカインにより活性化されたグリコーゲンsynthase kinase-3β(GSK-3β)により，

筋線維内のリン酸化タウ産生が増加することが判明した．sIBMでは，GSK-3βとリン酸化タウが共存しており，炎症過程とAβとの関連性が示唆された[15,43]．

12 sIBMのバイオマーカー

生検筋の病理学的検討の際のバイオマーカーとしては，RV，非壊死線維への細胞浸潤，筋細胞内へのβアミロイド，TDP-43，SMI-31，p62の沈着があげられる．近年，sIBM患者血清中に分子量43 kDaの自己抗体が見いだされた．Western blotを用いた検討では，当初anti-IBM43といわれたこの自己抗体はsIBMの診断に関して感度50％，特異度100％であった．その後の検討で，この43 kDaは，cytoplasmic 5′-nucleotidase 1A(cN1A; NT5C1A)であることが判明し，抗cN1A抗体を用いたsIBMの病理学的検討では，cN1Aは筋核の周囲および，RVの周囲に局在し，TDP-43とは共局在しなかった．臨床的には，本抗体陽性例は陰性例に比べ歩行の際に補助具が必要な症例が多く，嚥下障害を訴えたり，肺活量が低下している症例が多いとの報告もある[44]．抗cN1A抗体は，SLEやSjögren症候群のような他の自己免疫性疾患でも陽性となることがあるが[45]，IBMの自己免疫性疾患の要素と変性疾患の要素を介在するものとして注目されている[46]．

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その他の炎症性ミオパチー

増殖性筋炎

Proliferative myositis

村田顕也

Key words：神経節細胞様巨細胞，線維芽細胞様紡錘細胞，偽性肉腫，増殖性筋膜炎

1. 概　念

本疾患は，1960年にKernによって初めて報告された[1]．筋炎という名称がついているが，病変の主体は①神経節細胞様/横紋筋芽細胞様の巨細胞と②線維芽細胞様の紡錘細胞が非腫瘍性に増殖する偽性肉腫であり，筋線維は萎縮や局所的な虚血に伴う二次的な変化を示すのみである．以前は骨形成筋炎の一亜型と考えられたこともあったが，最近では，独立した疾患単位とされている．中高年齢層(特に45歳以上)に好発し，若年者例はまれである[2]．性差は認めない．

2. 病　因

不明である．外傷の既往が本疾患の発症要因とされているが，外傷や過剰な活動の既往を有さない症例でも発症しており，明確ではない．

3. 病　態

腫瘤は，上肢，下肢，腹部，体幹，胸鎖乳突筋[3]，時には下顎，頸部[4,5]，咬筋[6]，舌[7]の筋内に痛みを伴いながら急速(多くは2-3週間以内)に増大し，時には腫瘤が，数日のうちに倍増することもある．好発部位は肩と前腕と体幹である．腫瘤は，直径1-6cm(平均3.8cm)で孤立しており，可動性は良好で，上部を覆う皮膚との癒着も認められない．腫瘤形成に先立って，罹患部の皮膚の腫脹・発赤を呈した症例がまれに報告されている．

4. 診断と鑑別診断

病変部位は，MRI T1強調画像で低信号または等信号を呈し，T2強調画像では高信号を呈する．造影剤を用いれば，全般的に造影される．FDG-PETでは，腫瘤部は高集積を示す[8]．これらの画像所見は病変部位の同定には有益であるが，悪性腫瘍との鑑別は困難で，診断には部分切除による病理学的検討が必要である[9]．筋腫瘤は，肉眼的には灰色もしくは白色の固い腫瘤で，周辺組織との癒着はないが，正常な筋組織との境界は不明瞭である．罹患筋が小さい筋肉の場合は，筋肉全体が肥厚したように増生するが，大きな筋肉の場合は，表層に形成される．

本疾患の中核病理像は，①epimysium，endomysium，perimysiumに境界不明瞭に増殖する線維芽細胞様紡錘細胞と②神経節細胞様(ganglion-like cell)/横紋筋芽細胞類似の大型の好塩基性巨細胞である．線維芽細胞様細胞は，紡錘形の細胞で集合状ないし散在的に出現し線維性結合組織増生の主体をなしている．異形成は認めず，免疫染色では，vimentin陽性を示すが，一部はsmooth muscle actinが陽性と報告されている[7]．巨細胞は，群または巣状に分布しており，円形もしくは多角形を呈し，好塩基性に染まる細胞質から成り立っている．核は，円形ないし楕円形で，核クロマチンは軽度増加しているが，その分布は均等で微細顆粒状である．大きな核小体がみられるものや多核の細胞も認められる．巨細胞の由来については議論があり筋芽細胞由来という考え方もある．しかし，巨細

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胞と線維芽細胞との中間型の細胞が存在したり，巨細胞の多くが筋線維内でなく，増殖した結合織に存在することから，現在では，非定型的な形や大きさを有した線維芽細胞と考えられている．近年の免疫染色では，desmin，smooth muscle actinは陰性でvimentinのみ陽性を示し今後の検討が必要である[7]．一方，炎症性変化は軽度で，リンパ球や組織球といった細胞が血管周囲や病変周囲の筋組織に存在している程度であり，細胞浸潤が著明というわけではない．症例によっては，単一の筋線維が壊死・再生を呈している場合もあるが，筋線維がprimaryに障害されているわけではない．増殖した結合織と残存した筋線維によって病変部位がchecker-board(格子模様)のように見えることもある．

軟部組織に形成される偽性肉腫として，本症以外に①結節性筋膜炎と②増殖性筋膜炎が鑑別上重要である．結節性筋膜炎の腫瘤は線維芽細胞の増殖のみで神経節様または線維芽細胞様巨細胞は存在しない．増殖性筋膜炎は，病変の主体が筋肉でなく皮下の脂肪組織や浅在筋膜に生じた場合に診断されるが，本疾患と同一の疾患とする報告もある[10]．神経節細胞様/筋芽細胞様の好塩基性の巨細胞が存在するため，横紋筋肉腫・線維芽肉腫・脂肪肉腫と診断されることが多い．

5. 治療と予後

本疾患は，予後良好な疾患であり，罹病期間の差異はあるものの(1-16年)，自然軽快する．悪性腫瘍との鑑別のため生検または除去が行われることが多いが，再発例はなく，継続的な外科的処置は不要である．局所の再発や全身の脱力や全身性疾患への移行の報告はなく，確定診断がついたら，経過観察のみで十分である．

6. そ の 他

以上述べたように本疾患は線維芽細胞様紡錘細胞と神経節細胞様/筋芽細胞様巨細胞による肉芽腫様増殖性病変であり，現在では非腫瘍性と考えられている．近年，trisomy 2の報告がなされ，症例によっては，転座や染色体の部分欠損が起こり，融合遺伝子の形成や，遺伝子欠損を引き起こしている可能性も否定できない[11]．組織学的には非腫瘍性に見えても，本質的には腫瘍，またはその前段階の可能性もあり，今後も遺伝子学的見地からの再検討が必要と考えられる．

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A CARRIER WITH *DE NOVO* MUTATION IN THE DYSTROPHIN GENE WHOSE MYOPATHIC SYMPTOMS BECAME SERIOUSLY PROGRESSIVE AFTER PREGNANCY AND DELIVERY

A minority of women with mutations in the dystrophin gene show symptoms and are referred to as manifesting carriers. Hoogerwaard *et al.* reported that 22% of them had symptoms, and 17% had muscle weakness, varying from mild to severe.[1,2] Recently, we encountered a manifesting carrier whose muscle symptoms deteriorated critically after pregnancy and delivery.

The patient was a 30-year-old woman with hyperCKemia and significant muscle weakness. When she visited another hospital at age 27 complaining of fatigue, a blood test revealed mildly elevated transaminases and hyperCKemia (2,150 IU/L). Although a full diagnostic work-up was done, her disease was not identified. Otherwise, she had been without serious problems before pregnancy at age 28. Due to a fetal breech presentation, Cesarean section was carried out at week 37 of gestation. Standard spinal anesthesia was used, and a healthy girl was born. After delivery, the patient was first noted to have an abnormal gait. At the same time she began to feel muscle weakness in her right arm. While taking care of her baby, the gait disturbance and difficulties experienced in performing other daily tasks increased rapidly.

One year after delivery she visited us for the first time. She exhibited calf muscle hypertrophy and a Gowers sign, but did not have scapular winging. She exhibited decreased tendon reflexes in her right upper and bilateral lower extremities. In particular, the reflexes in proximal muscles were absent. Abnormal laboratory data were as follows: serum aspartate aminotransferase 59 (normal 10–40) IU/L; alanine aminotransferase 85 (5–40) IU/L; serum creatine kinase (CK) 2,058 (45–176) IU/L; and CK-MB 84 (6–18) IU/L. Echocardiography and electrocardiography did not show apparent abnormalities. Specifically, she did not have left ventricle dilation, a frequent finding

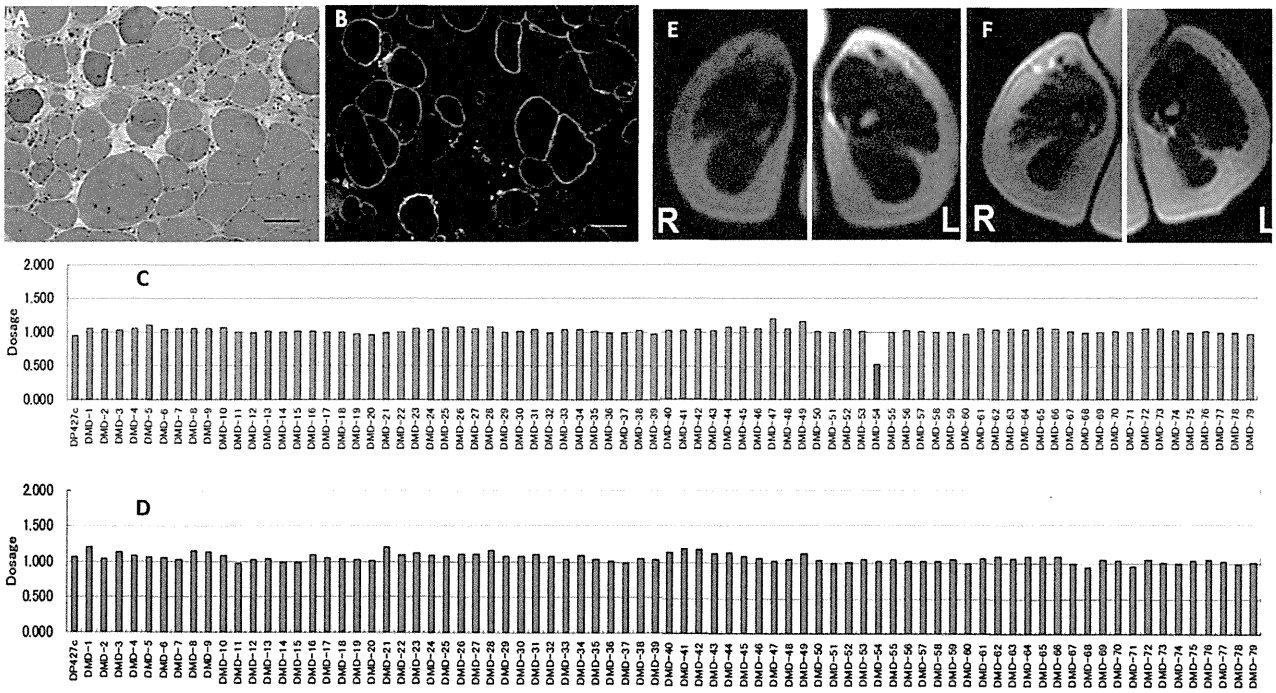

FIGURE 1. (A) Hematoxylin and eosin stain of muscle histology reveals variation in fiber size, hypertrophic fibers, internal nuclei, opaque fibers, and slight fibrosis. Fiber splitting is also seen. **(B)** Dystrophin immunostaining shows patchy membrane staining. Scale bar = 100 μm. **(C, D)** Multiplex ligation-dependent probe amplification (MLPA) analyses for the patient **(C)** and her mother **(D)** reveal that the patient has a *de novo* deletion mutation in exon 54, but her mother does not. X-axis: exon number; Y-axis: gene dose is quantified as ratio of signal intensity to normal control signal intensity. Thus, 1.000 means both dystrophin alleles are present. **(E, F)** T2-weighted MRI of upper limbs, at age 30 years **(E)** and at age 34 years **(F)**. The MRIs show rapid deterioration in the same skeletal muscles over 4 years. R and L stand for right and left upper limbs, respectively.

in Duchenne/Becker muscular dystrophy carriers.[3] There were no abnormalities in shoulder girdle muscles. On concentric needle EMG, motor unit potential amplitudes were <200 μV. On routine histological study of a muscle biopsy from her right biceps brachii, obvious dystrophic changes were found, but no there was no fibrosis (Fig. 1A). Immunohistochemical staining with anti-dystrophin antibodies revealed a mosaic distribution of positive fibers (approximately 50%) (Fig. 1B). Genetic analysis proved that this carrier's mutation was an exon 54 deletion resulting in an out-of-frame mutation in 1 allele (Fig. 1C). On the other hand, her mother had no mutation (Fig. 1D).

Her muscle strength deteriorated rapidly and, by 2 years after her baby's birth, she could not walk without a cane. Follow-up limb MRI study 4 years after diagnosis also confirmed the rapid progression of muscle atrophy (Fig. 1E and F).

Reports of progressive weakness in dystrophinopathy associated with pregnancy are rare.[4] It should be noted that, in the patient we have described, pregnancy and delivery were probably a critical factor in the deterioration of her myopathic symptoms. Immunohistological analysis and multiplex ligation-dependent probe amplification (MLPA) gene analysis[5–9] can be used to counsel patients who are considering pregnancy.

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Clinicopathological features of the first Asian family having vocal cord and pharyngeal weakness with distal myopathy due to a *MATR3* mutation

Distal myopathy is a clinically and pathologically heterogeneous disorder that selectively or disproportionately affects distal muscles of the upper and/or lower limbs [1]. An adult-onset, progressive autosomal dominant distal myopathy that is frequently associated with dysphagia and dysphonia, vocal cord and pharyngeal weakness (VCPDM/MPD2) was recently discovered in a North American and a Bulgarian family; its causative agent being a missense mutation in the *matrin-3 (MATR3)* gene [2,3]. Still, VCPDM remains a fairly rare disease that has only been reported in two families worldwide so far.

According to a previous report on VCPDM, muscle biopsy performed on the quadriceps or gastrocnemius muscles revealed chronic non-inflammatory myopathy with subsarcolemmal rimmed vacuoles (RV) and atrophic fibres, with denervation [2]. Pathologic changes were reported to be more severe in the gastrocnemius than in the quadriceps muscles. Electrophysiological studies have also shown some degree of combination of myogenic and neurogenic changes associated with VCPDM [2].

Here, we report the clinicopathological features of the first Asian family having VCPDM with a missense mutation in the *MATR3* gene. We also examined whether muscle pathology in patients with VCPDM shared histopathological characteristics with other myopathies with RV, including sporadic inclusion body myositis (sIBM), oculopharyngeal muscular dystrophy (OPMD), glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase (GNE) myopathy, and valosin-containing protein (VCP) myopathy.

Two Japanese half sisters were examined and summarized in Table 1. Their father noticed a disturbance in his gait in his forties and was dependent on a powered wheelchair in his sixties. He gradually developed respiratory problems and eventually underwent a tracheostomy with mechanical ventilation. He died of respiratory failure at 73.

Case 1, a 44-year-old woman experienced difficulty in ambulation and developed dysphagia of liquid and solids. Upon admission to our hospital, her neurological examination revealed dysphagia and dysarthria, while facial weakness and tongue atrophy were not observed. Moderate muscle weakness was detected in the neck flexor, and mild weakness without fasciculation was observed in the iliopsoas, hamstring, and tibialis anterior muscles. Touch and pinprick sensations were reduced in the distal upper and lower limbs, while vibration and position sense remained intact. Tendon reflexes, especially in the patella tendons, were generally weak.

Case 2, a 68-year-old woman (half sister of the patient in case 1) experienced difficulty in swallowing at age 63 and developed speech difficulty and finger weakness at age 65. Dysphagia and dysarthria progressed gradually until three months before hospital admission. After developing dyspnoea and somnolence, she was admitted to the hospital. Because of her respiratory dysfunction type 2 (PaO_2 50.5 mmHg, $PaCO_2$ 76.7 mmHg) diagnosis, she was treated with non-invasive positive pressure ventilation. Neurological examination showed dysphagia and nasal voice, despite there being no obvious facial weakness or tongue atrophy. Wasting was observed in the bilateral thenar, hypothenar, and first dorsal interossei muscles without fasciculation. The muscle weakness decreased moderately in the wrist extensors, iliopsoas, and extensor hallucis longus muscles and mildly in the deltoid, hamstring, and tibialis anterior muscles. Touch, pinprick, vibration, and position sensations remained intact but slight dysesthesia was present in the toe tips. Tendon reflexes were absent, except of a markedly decreased patella tendons reflex. Both cases of the patients did not fulfil diagnostic criteria of ALS because they lacked upper motor neurone signs.

After obtaining informed consent from patients and approval from a local ethics committee, genomic DNA was extracted from the peripheral blood samples for both patients. We conducted exome-sequencing to determine

Table 1. Summary of clinical data

	Case 1	*Case 2*
Age at biopsy	44	68
Age at onset	40	63
Gender	F	F
Distal weakness		
Legs	+	+
Hands	−	+
Shoulder weakness	−	+
Swallowing dysfunction	+	+
Vocal dysfunction	−	+
Respiratory dysfunction	−	+
CK (U/L, normal ranges: 45–176)	241	81
EMG	Myogenic + neurogenic	Myogenic + neurogenic
NCS	Axonal degeneration type sensorimotor polyneuropathy	Axonal degeneration type motorsensory polyneuropathy
Abnormal lesions in skeletal MRI	Gluteus, quadriceps, hamstring	Paraspinal, gluteus
%FVC (%)	58.9	36.0

the causative mutation for each patient. Exonic sequences were enriched using a SureSelect V4+UTR (Agilent) and subjected to massively parallel sequencing using Illumina Hiseq2000 (100 bp paired-end). Burrows Wheeler Aligner [4] and Samtools [5] were used for alignment and variation detection. It revealed a missense mutation in the *MATR3* gene: p.S85C (c.254C>G), which was exactly the same mutation as described in the only two previous families of VCPDM with a missense mutation in the *MATR3* gene by Senderek *et al.* [3]. Sanger sequencing confirmed this mutation for both cases.

In case 1, the patient underwent biopsy from the left biceps brachii muscle. Haematoxylin and eosin (HE) staining showed a severe fatty change in myofibres of various sizes (Figure 1**a**). Approximately 5% of myofibres presented myopathic changes with RV and internal nuclei (Figure 1**b**,**c**). Inflammatory cellular infiltrates were absent. Acid phosphatase staining showed weak activity consistent with lysosomal activity levels in the RV (Figure 1**d**). ATPase staining showed a predominance of type 1 fibres (Figure 1**e**,**f**). Neither upregulation of major histocompatibility complex (MHC) class I nor cytochrome c oxidase (COX)-negative muscle fibres was observed (data not shown).

In case 2, the patient underwent biopsy from the right biceps brachii muscle. HE staining showed that 1–2% of myofibres presented myopathic changes with RV and internal nuclei (Figure 1**g**,**h**). Inflammatory cellular infiltrates were not observed. Acid phosphatase staining showed no activity (Figure 1**i**). Interestingly, ATPase staining revealed a fibre-type grouping with an increase in type 2 fibres, indicating neurogenic changes (Figure 1**j**–**l**). The specimens showed no upregulation of MHC class I or COX-negative fibres (data not shown).

Electron microscopy of samples from case 1 demonstrated abundant autophagic vacuoles in degenerative myofibres (Figure 1**m**,**n**). As far as we could observe, we found no intranuclear aggregates (Figure 1**n**).

Next, we asked whether myopathic changes associated with VCPDM shared similar histopathological characteristics with myopathies with RV including sIBM, OPMD, GNE myopathy and VCP myopathy. The study was approved by the Ethics Committee of the Kumamoto University Hospital. Recent studies have shown that p62 is the best histological diagnostic marker for sIBM [6–9]. Therefore, we performed immunofluorescence staining using mouse anti-p62/SQSTM1 (1:250; Medical & Biological Laboratories, Nagoya, Japan) and rabbit anti-MATR3 (1:250; Bethyl Laboratories, Montgomery, TX, USA) antibodies. In healthy control subjects, p62 was not detected in normal muscle fibres (data not shown). Immunohistochemical analyses of p62 revealed its sarcoplasmic aggregates in 10–20% of the myofibres in patients with VCPDM (Figure 2**a**,**e**). Substantial immunoreactivity for p62 was observed in myofibres of patients with sIBM (Figure 2**i**), OPMD (Figure 2**m**) as well as GNE myopathy (Figure 2**q**) and VCP myopathy (Figure 2**u**). In healthy control subjects, all myonuclei stained for MATR3 (data not shown).

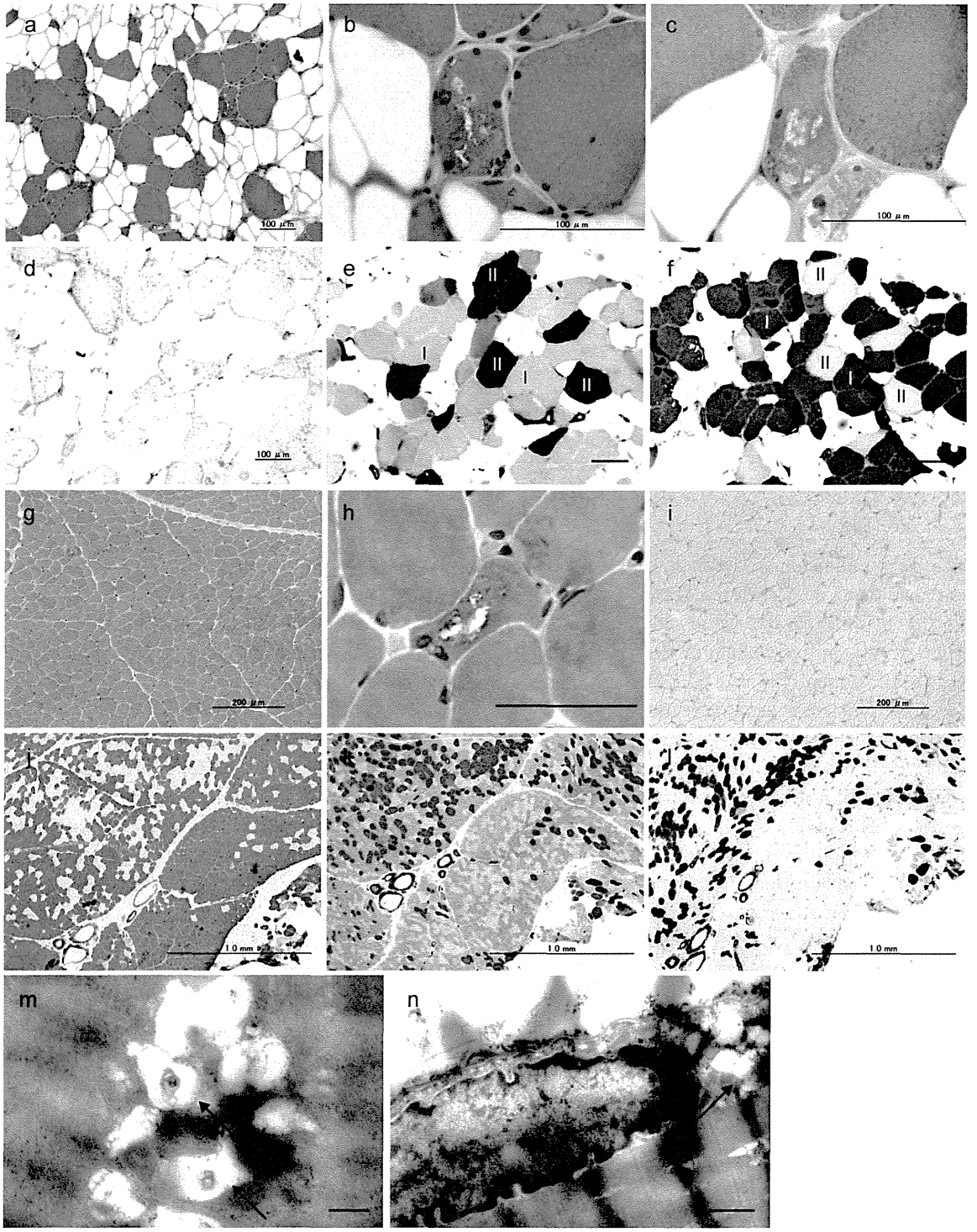

Figure 1. Muscle histology for the biopsy samples of VCPDM case 1 and 2. (**a–f**) VCPDM case 1: (**a**, **b**) Haematoxylin and eosin (HE) staining at lower (**a**) and higher (**b**) magnifications. (**c**) Modified Gomori-trichrome staining. (**d**) Acid phosphatase staining. (**e**, **f**) ATPase staining at pH 10.6 (**e**), and pH 4.2 (**f**). I and II indicate type 1 and 2 fibres, respectively. Scale bars = 100 μm. (**g–j**) VCPDM case 2: (**g**, **h**) HE staining at lower (**g**) and higher (**h**) magnifications. (**i**) Acid phosphatase staining. (**j–l**) ATPase staining at pH 10.7 (**j**), pH 4.5 (**k**) and pH 4.2 (**l**). Scale bars = 200 μm (**g**, **i**), 50 μm (**h**) and 1.0 mm (**j–l**). (**m**, **n**) Electron microscopic analysis of samples from VCPDM case 1. Arrows indicate autophagic vacuoles. Scale bars = 500 nm (**m**), 800 nm (**n**).

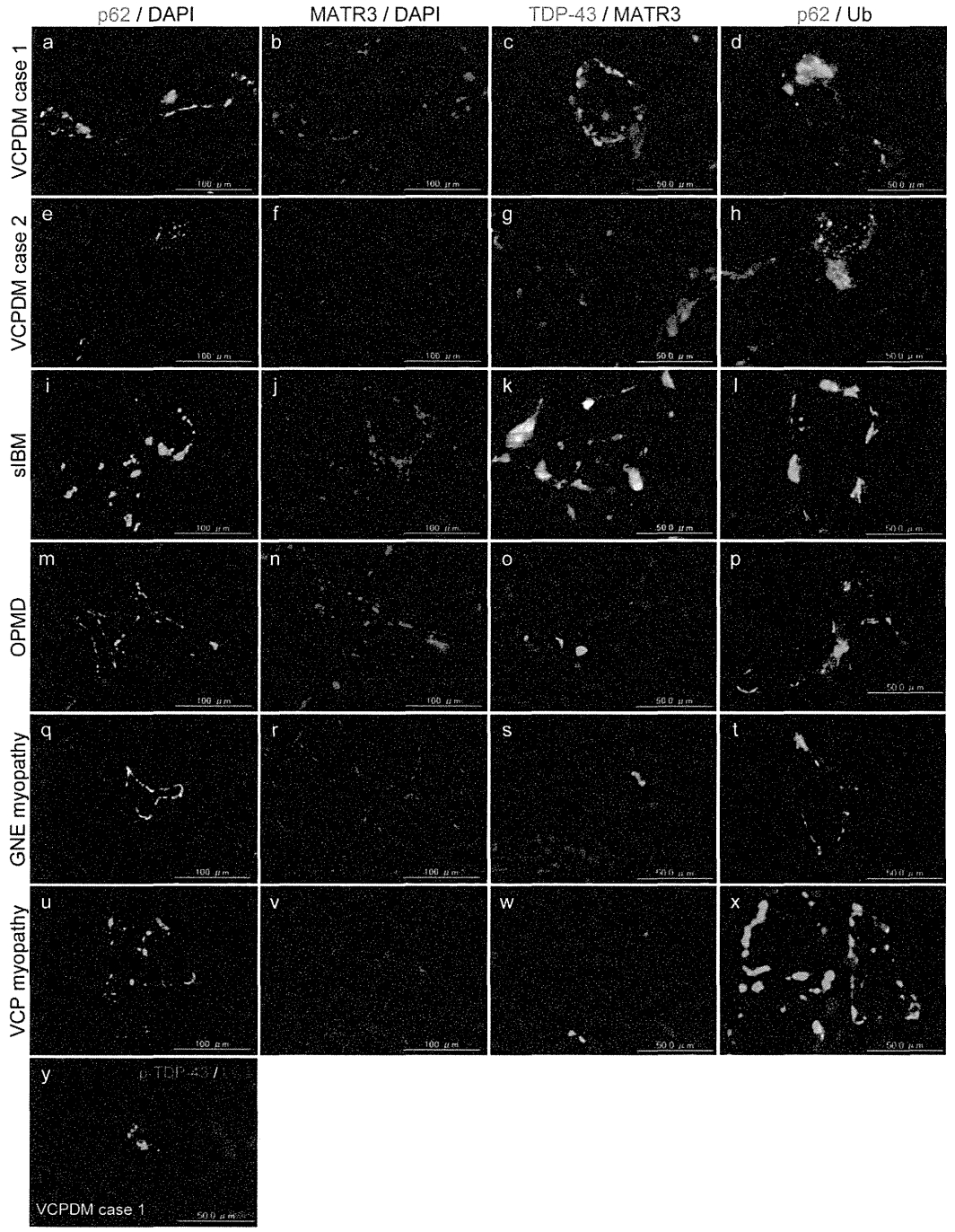

Figure 2. Immunofluorescence studies for proteins related to myopathies with rimmed vacuoles. Immunofluorescence study of p62 (green; **a**, **e**, **i**, **m**, **q**, **u**) and MATR3 (red; **b**, **f**, **j**, **n**, **r**, **v**) in identical specimens from VCPDM case 1 (**a**, **b**), case 2 (**e**, **f**), sIBM (**i**, **j**), OPMD (**m**, **n**), GNE myopathy with homozygous p.V572L mutation (**q**, **r**), and VCP myopathy with heterozygous p.A232E mutation (**u**, **v**). Double immunofluorescence study of TDP-43 (green) and MATR3 (red) in VCPDM case 1 (**c**), case 2 (**g**), sIBM (**k**), OPMD (**o**), GNE myopathy (**s**) and VCP myopathy (**w**). Double immunofluorescence study of p62 (green) and ubiquitin (red) in VCPDM case 1 (**d**), case 2 (**h**), sIBM (**l**), OPMD (**p**), GNE myopathy (**t**) and VCP myopathy (**x**). Double immunofluorescence study of phosphorylated TDP-43 (green) and LC-3 (red; 1: 500; Medical & Biological Laboratories, Nagoya, Japan) in VCPDM case 1(**y**). Immunolabelled proteins were visualized using anti-mouse immunoglobulin antibody-conjugated Alexa Fluor 488 or anti-rabbit immunoglobulin antibody-conjugated Alexa Fluor 594 (1:200; Life Technologies Corporation, Carlsbad, CA, USA). Scale bars = 100 μm (**a**, **b**, **e**, **f**, **m**, **n**, **q**, **r**, **u**, **v**) and 50 μm (**c**, **d**, **g**, **h**, **k**, **l**, **o**, **p**, **s**, **t**, **w**, **x**, **y**). Nuclei were stained with 4′, 6-diamidino-2-phenylindole (blue).

Table 2. Semi-quantitative analysis for immunohistochemistry

	p62	*MATR3*	*TDP-43*	*Ubiquitin*
VCPDM Case 1	++, aggregates	+, granular or loss of nuclear staining	++, aggregates	+, granular
VCPDM Case 2	++, aggregates	+, granular or loss of nuclear staining	±, diffuse	+, granular
sIBM	++, aggregates	±, granular	++, aggregates	+, granular
OPMD	++, aggregates	±, granular	++, aggregates	+, granular
GNE myopathy	++, aggregates	±, granular or loss of nuclear staining	+, aggregates	+, granular
VCP myopathy	++, aggregates	±, granular or loss of nuclear staining	++, aggregates	+, granular

VCPDM, vocal cord and pharyngeal weakness with distal myopathy; sIBM, sporadic inclusion body myositis; OPMD, oculopharyngeal muscular dystrophy. –, no positive cells; ±, occasional positive cells; +, moderate numbers of positive cells; ++, frequent numbers of positive cells.

Immunohistochemical analysis of MATR3 demonstrated sarcoplasmic granular staining in p62-positive degenerating myofibres for case 1 (Figure 2**b**). Some myonuclei showed a loss in immunoreactivity for MATR3 (Figure 2**b**). In case 2, some myonuclei presented immunoreactivity loss for MATR3 without sarcoplasmic staining (Figure 2**f**). Sarcoplasmic granular staining for MATR3 was observed in some p62-positive degenerating myofibres of patients with sIBM (Figure 2**j**), OPMD (Figure 2**n**), and GNE (Figure 2**r**) and VCP (Figure 2**v**) myopathies. Notably, most myonuclei remained strongly reactive to MATR3 in sIBM and OPMD, (Figure 2**j**,**n**) whereas some myonuclei showed a loss in immunoreactivity for MATR3 in GNE (Figure 2**r**) and VCP (Figure 2**v**) myopathies.

We then examined whether other proteins involved in RV-related myopathies accumulated in the myofibres of patients with VCPDM. Previous studies have shown frequent accumulation of TAR DNA-binding protein 43 kDa (TDP-43) in sarcoplasmic granules within degenerating myofibres of patients with sIBM (Figure 2**k**), OPMD (Figure 2**o**) and GNE (Figure 2**s**) and VCP (Figure 2**w**) myopathies. Within myofibres with TDP-43-immunoreactive sarcoplasmic aggregates, nuclei were less immunoreactive for TDP-43 in patients with sIBM (Figure 2**k**). An immunohistochemical analysis using mouse anti-TDP-43 (1: 250; ProteinTech Group, Chicago, IL, USA) antibody demonstrated the presence of its sarcoplasmic aggregates (~10%) in myofibres for Case 1 (Figure 2**c**) and diffuse cytoplasmic staining in myofibres for Case 2 (Figure 2**g**). In myofibres with TDP-43-positive aggregates in Case 1, myonuclei were less immunoreactive for both TDP-43 and MATR3, although both proteins did not necessarily colocalize (Figure 2**c**). Interestingly, some TDP-43-positive granules were immunoreactive for mouse anti-phosphorylated TDP-43 (pS409/410) (1: 3000; Cosmo Bio, Tokyo, Japan) antibody (Figure 2**y**).

Because a deficit in protein degradation machinery is suspected to be one of the pathophysiological mechanisms underlying RV-related myopathies, we investigated the involvement of ubiquitin in the myofibres of patients with VCPDM, using rabbit anti-ubiquitin (1: 200; Dako) antibody. In these patients, immunohistochemistry for ubiquitin showed sarcoplasmic granular staining mainly in p62-positive fibres (Figure 2**d**,**h**). Sarcoplasmic granular staining for ubiquitin was also observed in sIBM (Figure 2**l**), OPMD (Figure 2**p**) as well as GNE (Figure 2**t**) and VCP (Figure 2**x**) myopathies. Expression profiles are summarized in Table 2.

We herein reported clinicopathological features of the first Asian family having VCPDM with a missense mutation in the *MATR3* gene: p.S85C (c.254C>G), which was a sole mutation that has been described in the previous cases with VCPDM. Collectively, our results showed intrafamilial variation including the presentation of motorsensory neuropathy. We identified the histopathological characteristics of VCPDM: myopathic changes with RV but no inflammatory infiltrate, neurogenic changes, diffuse sarcoplasmic distribution of MATR3 and/or loss of nuclear staining, and other histological features common to RV-myopathies, such as accumulation of p62, TDP-43 and ubiquitin.

According to a previous report on the clinical features of VCPDM [2], muscle weakness is exhibited asymmetrically in the feet and ankles and/or the hands. The distribution of weakness in the lower limbs has been more affected in the peroneal muscles than in the gastrocnemius muscles. Weakness in the upper limbs occurs more often in the finger extensors and abductor pollicis brevis

(APB), and to lesser extent in the deltoid muscles. While vocal cord and pharyngeal weakness can be present at the onset of the distal weakness, some patients show neither vocal cord dysfunction nor problems swallowing. Our skeletal muscle MRI data indicated that the quadriceps muscles were relatively spared. Of note, the sparing of the vastus lateralis was described in another distal myopathy with RV, such as GNE myopathy [10], and the similarity might suggest the common pathogenesis between the both diseases.

Muscle histology in patients with VCPDM has previously revealed chronic non-inflammatory myopathy in addition to the presence of RV, usually in subsarcolemmal as well as atrophic fibres [2]. However, the specific characteristics of VCPDM have still not been conclusively determined. TDP-43 has been identified as a major component protein of ubiquitin-positive inclusions in the brains of patients with frontotemporal lobar degeneration with ubiquitin-positive inclusions and in the spinal anterior horns of patients with amyotrophic lateral sclerosis (ALS) [11,12]. TDP-43-positive granules have been observed not only in sIBM but also in other vacuolar myopathies such as OPMD, and VCP and GNE myopathies [7,13–17]. Our observation of TDP-43-positive granules in VCPDM suggests that the presence of TDP-43-positive aggregates may be a common phenomenon among myopathies associated with RV [8,13,14,17,18].

MATR3 is a component of the nuclear matrix and thought to be associated with the protein machinery for transcription, RNA splicing and DNA replication [3]. To date, the mutation of p.S85C (c.254C>G) in the *MATR3* gene is a sole mutation described in the previous cases with VCPDM. Recent exome-sequencing study has revealed mutations in the *MATR3* gene in some of ALS kindreds [19]. Interestingly, the report included one of the families harbouring the S85C mutation that had been originally described as having myopathy due to the *MATR3* mutation [3], and reclassified the condition as slowly progressive familial ALS. However, we provide definite evidence that the S85C *MATR3* mutation actually induced distal myopathy with minor neurogenic features. Taken together with these observations, the *MATR3* mutation can indeed cause wide-ranged phenotypes from inclusion body myopathy to motor neurone disease.

Although MATR3 is a multifunctional protein [19], the effect of the mutation on structure and function of MATR3 protein remains unsolved. Our observation of the sarcoplasmic accumulation of p62, TDP-43, and ubiquitin suggests a deficit in protein degradation, possibly due to ubiquitin proteasome system dysfunction and/or autophagy. Furthermore, the findings that immunoreactivity loss for MATR3 in the myonuclei was related with its sarcoplasmic staining might suggest that the mutation in the *MATR3* gene interferes directly or indirectly with the protein localization resulting in loss-of-function. The dysfunction of MATR3 by its mutation would possibly lead to a modification in gene expression related to abnormal chromatin organization, deregulation of nuclear mRNA export, abnormal pre-mRNA splicing, or nuclear proteome alterations in skeletal muscles. As *MATR3* knockdown caused deficit in the machinery for DNA damage response and cell cycle [20], such a nuclear dysfunction might be involved in VCPDM pathogenesis. Further investigation and establishing an understanding of the *MATR3* mutation in transgenic animals will be necessary to elucidate the pathophysiological mechanisms underlying myofibre degeneration and neuropathic change.

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Authors' contribution

SY and AM: conception, design, acquisition of data, analysis and interpretation. YN, RK, NT, TN, YM, HU, SI, YH, AH, IH, SM and JY: acquisition of data. MU, HT and ST: acquisition of data and critical revision of the manuscript for important intellectual content. AY: analysis and interpretation, critical revision of the manuscript for important intellectual content and study supervision.

Conflict of interest

The authors declare that they have no conflict of interest.

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REVIEW Open Access

Genotype-phenotype relationship in hereditary amyotrophic lateral sclerosis

Satoshi Yamashita* and Yukio Ando

Abstract

Amyotrophic lateral sclerosis (ALS) is the most common adult-onset motor neuron disease. It is characterized by neuronal loss and degeneration of the upper motor neurons (UMNs) and lower motor neurons (LMNs), and is usually fatal due to respiratory failure within 3–5 years of onset. Although approximately 5–10 % of patients with ALS have an inherited form of the disease, the distinction between hereditary and apparently sporadic ALS (SALS) seems to be artificial. Thus, genetic factors play a role in all types of ALS, to a greater or lesser extent. During the decade of upheaval, the evolution of molecular genetics technology has rapidly advanced our genetic knowledge about the causes of ALS, and the relationship between the genetic subtypes and clinical phenotype. In this review, we will focus on the possible genotype-phenotype correlation in hereditary ALS. Uncovering the identity of the genetic factors in ALS will not only improve the accuracy of ALS diagnosis, but may also provide new approaches for preventing and treating the disease.

Keywords: Amyotrophic lateral sclerosis (ALS), Genotype, Phenotype, Sporadic ALS (SALS), Familial ALS (FALS)

Introduction

Amyotrophic lateral sclerosis (ALS) is the most common adult-onset motor neuron disease. It is characterized by progressive neuronal loss and degeneration of the upper motor neurons (UMNs) and lower motor neurons (LMNs). The demise of motor neurons causes the central nervous system (CNS) to lose the ability to control voluntary muscle movement, eventually resulting in death due to respiratory failure in the later stages of the disease.

The cause of ALS remains an enigma. However, approximately 5–10 % of patients with ALS have an inherited form of the disease. During the decade of upheaval, the evolution of molecular genetics technology has rapidly advanced our knowledge about the genetic causes of ALS. Familial ALS (FALS) has been attributed to mutations in at least 24 different genes. Some mutations in FALS-related genes have been identified in patients with sporadic ALS (SALS). Because the initial symptoms of ALS vary across patients, a diagnosis of ALS can be established by excluding various diseases mimicking ALS. Smooth and reliable diagnosis is the first step in the good clinical management of patients with ALS. Therefore, genetic testing might be a helpful tool for diagnosing FALS as well as SALS with mutations in FALS-related genes.

It is important, but difficult, to predict which genes are most likely to be implicated in some patients with ALS. A diagnostic algorithm could improve the accuracy of a genetic explanation. Therefore, we review the possible genotype-phenotype relationship in ALS cases with mutations in the FALS-related genes. Uncovering the identity of the genetic factors in ALS will not only improve the accuracy of ALS diagnosis, but may also provide new approaches for preventing and treating the disorder.

Classification of hereditary ALS

Hereditary ALS can be transmitted as a dominant, recessive, or X-linked trait, but the most common type is an adult-onset disorder with autosomal dominant transmission. Autosomal recessive inheritance is rarer and frequently seen in patients with juvenile onset ALS, primary lateral sclerosis (PLS), or spastic paraplegia-like symptoms. X-linked dominantly inherited ALS is a rarely-observed condition, seen in families where male patients tend to show more severe phenotypes. We demonstrate the characteristic phenotypes in each type of FALS, and summarize them in Table 1.

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Table 1 The genotype and phenotype associated with familial ALS-related genes

Type	Gene	Mode of inheritance	Country	Age at onset (range)	Mean age at onset (years)	Initial symptoms	UMN	Cognitive impairment	Other features
ALS1	SOD1	AD, AR, de novo	Japan, Italy, Spain, Korea, UK, USA, Turkey, Sweden, Iran, Porland, Bulgaria, China, France, Germany, Denmark, Pakistan, Canada, and so on	6–94	48	LL > UL > bulbar	Positive (LMN dominant)	Very rare	Progressive muscular atrophy, progressive bulbar palsy, facial onset sensory motor neuronopathy (FOSMN) syndrome, vocal cord paralysis, cerebellar ataxia, sensory disturbance (vibration), autonomic dysfunction (incontinence, neurogenic bladder), lower back pain
ALS2	Alsin	AR	Tunisia, Saudi Arabia, Kuwait, Italy, Algeria, Hungary, Germany, The Netherlands, Pakistan, Bangladesh, Turkey, Japan, Portugal, France, Cyprus, China	1–11	2	LL, UL	Positive	None	Juvenile ALS, juvenile primary lateral sclerosis, infantile-onset ascending hereditary spastic paraplegia, generalized dystonia, cerebellar ataxia
ALS3	unknown	AD							
ALS4	SETX	AD	USA, Austria, Belgium, Italy, Afghanistan, China	1–73	19	LL > UL	Positive	None	Cerebellar ataxia, oculomotor apraxia (type 2), motor neuropathy, thin cervical spinal cord
ALS5	SPG11	AR	Italy, Turkey, Japan, Canada, Brazil	7–23	16	Bulbar, LL, UL	Positive	Rare (mental retardation)	Juvenile ALS, hereditary spastic paraparesis, autonomic dysfunction (incontinence)
ALS6	FUS	AD, AR, de novo	Belgium, Italy, Korea, UK, Japan, Turkey, Canada, France, USA, Germany	13–80	45	UL, bulbar > LL	Positive (LMN dominant)	Rare (mental retardation)	Progressive muscular atrophy, Parkinsonism, essential tremor, schizofrenia, learning disabilities
ALS7	unknown	AD							
ALS8	VAPB	AD	Brazil, UK, France (Japan), The Netherlands	18–73	44	UL, LL	Negative	None	Progressive muscular atrophy, progressive bulbar palsy, motor neuropathy, postural tremor, autonomic dysfunction (chronic intestinal constipation, sexual dysfunction)
ALS9	ANG	AD	The Neitherland, Ireland, Scotland, UK, USA, Sweden, Italy, France, Germany, China,	21–86	55	UL, LL, bulbar	Positive	FTD	Parkinsonism, progressive bulbar palsy
ALS10	TDP-43	AD, AR	Italy, France, UK, China, Germany, Turkey, USA, Belgium, Japan, Porland, Afghaistan, Canada	20–77	54	UL, LL, bulbar	Positive	FTD (rare)	Parkinsonism, chorea, progressive supranuclear palsy
ALS11	FIG4	AD	USA	29–77	55	Bulbar > UL, LL	Positive	None	Hereditary spastic paraparesis, primary lateral sclerosis, personality change
ALS12	OPTN	AD, AR	Japan, Italy, Turkey, The Netherlands, Denmark	24–83	51	Bulbar, UL, LL	Positive	FTD, AGD	Primary open angle glaucoma, parkinsonism, finger deformity, personality change, depression
ALS13	ATXN2	AD	USA, Belgium, the Netherlands, Canada, France, China, Germany, Switzerland, Italy, Turkey, Cuba	21–87	60	UL, LL	Positive	None	Cerebellar ataxia, corticobasal syndrome, Parkinsonism
ALS14	VCP	AD	Italy, USA, The Netherlands, Japan	36–68	48	LL > UL > bulbar	Positive	FTD	Paget's Disease, inclusion body myopathy

Table 1 The genotype and phenotype associated with familial ALS-related genes *(Continued)*

ALS15	UBQLN2	SD	USA, Australia, Canada, Italy, Turkey, Belgium, Germany, Bulgaria	M: 14-72, F: 16-77	44	UL, LL, bulbar	Positive	FTD	Primary lateral sclerosis, progressive bulbar palsy, relentlessly progressive choreoathetoid movements, spastic paralysis
ALS16	SIGMAR1	AD	Saudi Arabia	1-68	1	LL > UL	Positive	FTD (rare)	Juvenile ALS
ALS17	CHMP2B	AD	Denmark, the Netherlands	26-73	69	Bulbar, UL, LL, respiratory	Positive (LMN dominant)	FTD	Progressive muscular atrophy, parkinsonism
ALS18	PFN1	AD	Sephardic Jewish, Italy, USA, China, Belgium	33-63	53	Limb	N/A	N/A	
ALS19	ERBB4	AD	Japan, Canada	45-70	61	UL, bulbar, respiration	Positive	None	
ALS20	HNRNPA1	AD	N/A	N/A	N/A	N/A	N/A	FTD	Paget's Disease, inclusion body myopathy
ALS21	MATR3	AD	USA,UK, Italy, Taiwan	36-64	52	LL > UL, bulbar	Positive	FTD	Distal myopathy (inclusion body myopathy)
ALS-FTD1	C9ORF72	AD	Finland, Sardinia, Ireland, UK, Italy, USA,Canada, Germany, the Netherlands, Turkey, Israel, Australia, Japan	27–80	57	UL, LL, bulbar	Positive	FTD	Parkinsonism, cerebellar ataxia, psychosis,
ALS-FTD2	CHCHD10	AD	France, USA, Germany, Spain, Italy, Finland	35-73	56	Bulbar, UL, LL	Positive (LMN dominant)	FTD	Cerebellar ataxia, mitochondrial myopathy, deafness, neurogenic bladder, facial paresis, Parkinsonism
	TBK1	AD, de novo	Sweden, Denmark, Germany, France, Portugal	35-80	60	Bulbar, UL, LL, respiratory	Positive	FTD (50 %)	

AD, autosomal dominant; *AR*, autosomal recessive; *UL*, upper limb; *LL*, lower limb; *LMN*, lower motor neuron; *FTD*, frontotemporal dementia