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

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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 fibers, 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 dyspnea 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 fulfill diagnostic criteria of ALS because they lacked upper motor neuron 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 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. Hematoxylin and eosin (HE) staining showed a severe fatty change in myofibers of various sizes (Figure 1a). Approximately 5% of myofibers presented myopathic changes with RV and internal nuclei (Figure 1b, c). Inflammatory cellular infiltrates were absent. Acid phosphatase staining showed weak activity consistent with lysosomal activity levels in the RV (Figure 1d). ATPase staining showed a predominance of type 1 fibers (Figure 1e, f). Neither upregulation of major histocompatibility complex (MHC) class I nor cytochrome c oxidase (COX)-negative muscle fibers 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 myofibers presented myopathic changes with RV and internal nuclei (Figure 1g, h). Inflammatory cellular infiltrates were not observed. Acid phosphatase staining showed no activity (Figure 1i). Interestingly, ATPase staining revealed a fiber-type grouping with an increase in type 2 fibers, indicating neurogenic changes (Figure 1j–l). The specimens showed no upregulation of MHC class I or COX-negative fibers (data not shown).

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

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 fibers (data not shown). Immunohistochemical analyses of p62 revealed its sarcoplasmic aggregates in 10–20% of the myofibers in patients with VCPDM (Figure 2a, e). Substantial immunoreactivity for p62 was observed in myofibers of patients with sIBM (Figure 2i), OPMD (Figure 2m) as well as GNE myopathy (Figure 2q) and VCP myopathy (Figure 2u). In healthy control subjects, all myonuclei stained for MATR3 (data not shown). Immunohistochemical analysis of MATR3 demonstrated sarcoplasmic granular staining in p62-positive degenerating myofibers for case

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

We then examined whether other proteins involved in RV-related myopathies accumulated in the myofibers of patients with VCPDM. Previous studies have shown frequent accumulation of TAR DNA-binding protein 43kDa (TDP-43) in sarcoplasmic granules within degenerating myofibers of patients with sIBM (Figure 2k), OPMD (Figure 2o), and GNE (Figure 2s) and VCP (Figure 2w) myopathies. Within myofibers with TDP-43-immunoreactive sarcoplasmic aggregates, nuclei were less immunoreactive for TDP-43 in patients with sIBM (Figure 2k). 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 myofibers for Case 1 (Figure 2c) and

diffuse cytoplasmic staining in myofibers for Case 2 (Figure 2g). In myofibers 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 2c). Interestingly, some TDP-43-positive granules were immunoreactive for mouse anti-phosphorylated TDP-43 (pS409/410) (1: 3,000; Cosmo Bio, Tokyo, Japan) antibody (Figure 2y).

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 myofibers 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 fibers (Figure 2d, h). Sarcoplasmic granular staining for ubiquitin was also observed in sIBM (Figure 2l), OPMD (Figure 2p) as well as GNE (Figure 2t) and VCP (Figure 2x) 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 fibers [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 harboring 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 neuron 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. Since *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 myofiber degeneration

and neuropathic change.

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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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Table legends

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