Further analysis of SCA23 is required to investigate the association of clinical manifestations and mutations in Japan.

Financial disclosure/conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

Dr KS and MS collected samples, assessed patients clinically, performed data analysis and wrote the manuscript.

Dr. JM and MH performed molecular genetic analysis and wrote the manuscript.

Dr. MH, YI, and JG performed molecular genetic analysis and revised the manuscript.

Dr. ST and SK designed the study, collected samples, assessed all patients clinically and revised the manuscript.

Acknowledgments

This study was supported in part by the Ministry of Health, Labor, and Welfare of Japan [Research on intractable diseases (H23-017)] and the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Grants-in-Aid for Scientific Research, 24110518 and 26110721).

References

[1] Bakalkin G, Watanabe H, Jezierska J, Depoorter C, Verschuuren-Bemelmans C, Bazov I, et al. Prodynorphin mutations cause the neurodegenerative disorder spinocerebellar ataxia type 23. Am J Hum Genet 2010;87:593–603.
[2] Jezierska J, Stevanin G, Watanabe H, Fokkens MR, Zagnoli F, Kok J, et al. Identification and characterization of novel PDYN mutations in dominant cerebellar ataxia cases. J Neurol 2013;260:1807–12.
[3] Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence alignment/map format and SAM tools. Bioinformatics 2009;25:2078–9.
[4] Watanabe H, Saito Y, Terao S, Ando T, Kachi T, Mukai E, et al. Progression and prognosis in multiple system atrophy: an analysis of 230 Japanese patients. Brain 2002;125:1070–83.
[5] Watanabe H, Ito M, Fukatsu H, Senda J, Atsuta N, Kaga T, et al. Putaminal magnetic resonance imaging features at various magnetic field strengths in multiple system atrophy. Mov Disord 2010;25:1916–23.

Kazumasa Saigoh*
Department of Neurology, Kinki University Faculty of Medicine, Japan

Jun Mitsui
Department of Neurology, University of Tokyo, Graduate School of Medicine, Japan

Makito Hirano, Mitsuaki Shioyama, Makoto Samukawa
Department of Neurology, Kinki University Faculty of Medicines, Japan

Yaeko Ichikawa, Jun Goto, Shoji Tsuji
Department of Neurology, University of Tokyo, Graduate School of Medicine, Japan

Susumu Kusunoki
Department of Neurology, Kinki University Faculty of Medicine, Japan
E-mail address: kusunoki-tky@umin.ac.jp.

* Corresponding author. Department of Neurology, Kinki University Faculty of Medicine, 377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan. Tel.: +81 72 366 0221x3552; fax: +81 72 366 4846.
E-mail address: saigoh@k.email.ne.jp (K. Saigoh).

19 August 2014

Neuropathology and Applied Neurobiology (2015), **41**, 391–398 doi: 10.1111/nan.12179

Scientific correspondence

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).

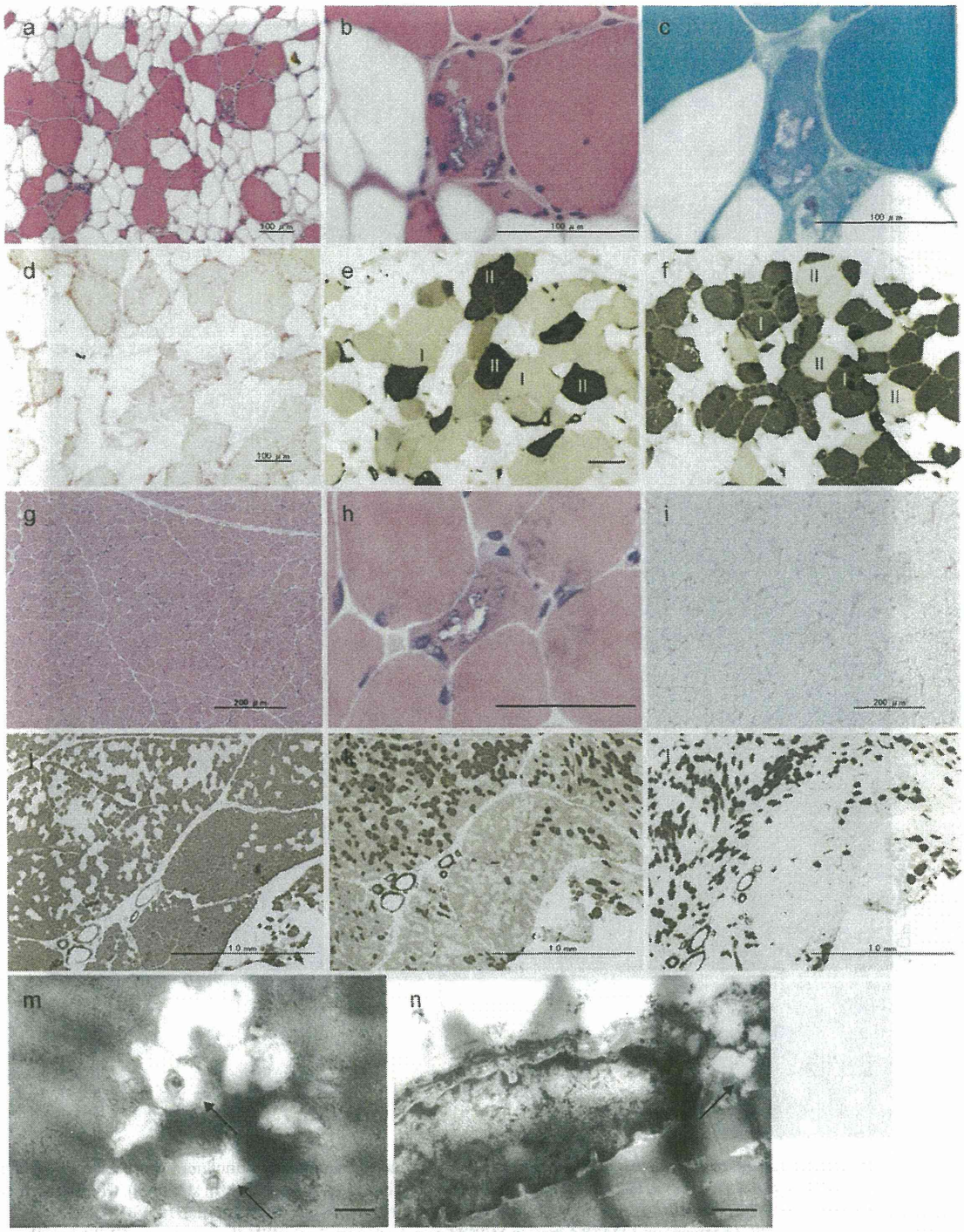

Figure 1. Muscle histology for the biopsy samples of VCPDM case 1 and 2. (**a**–**f**) VCPDM case 1: (**a**, **b**) Haematoxylin and eosin (HE) staining at lower (**a**) and higher (**b**) magnifications. (**c**) Modified Gomori-trichrome staining. (**d**) Acid phosphatase staining. (**e**, **f**) ATPase 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**).

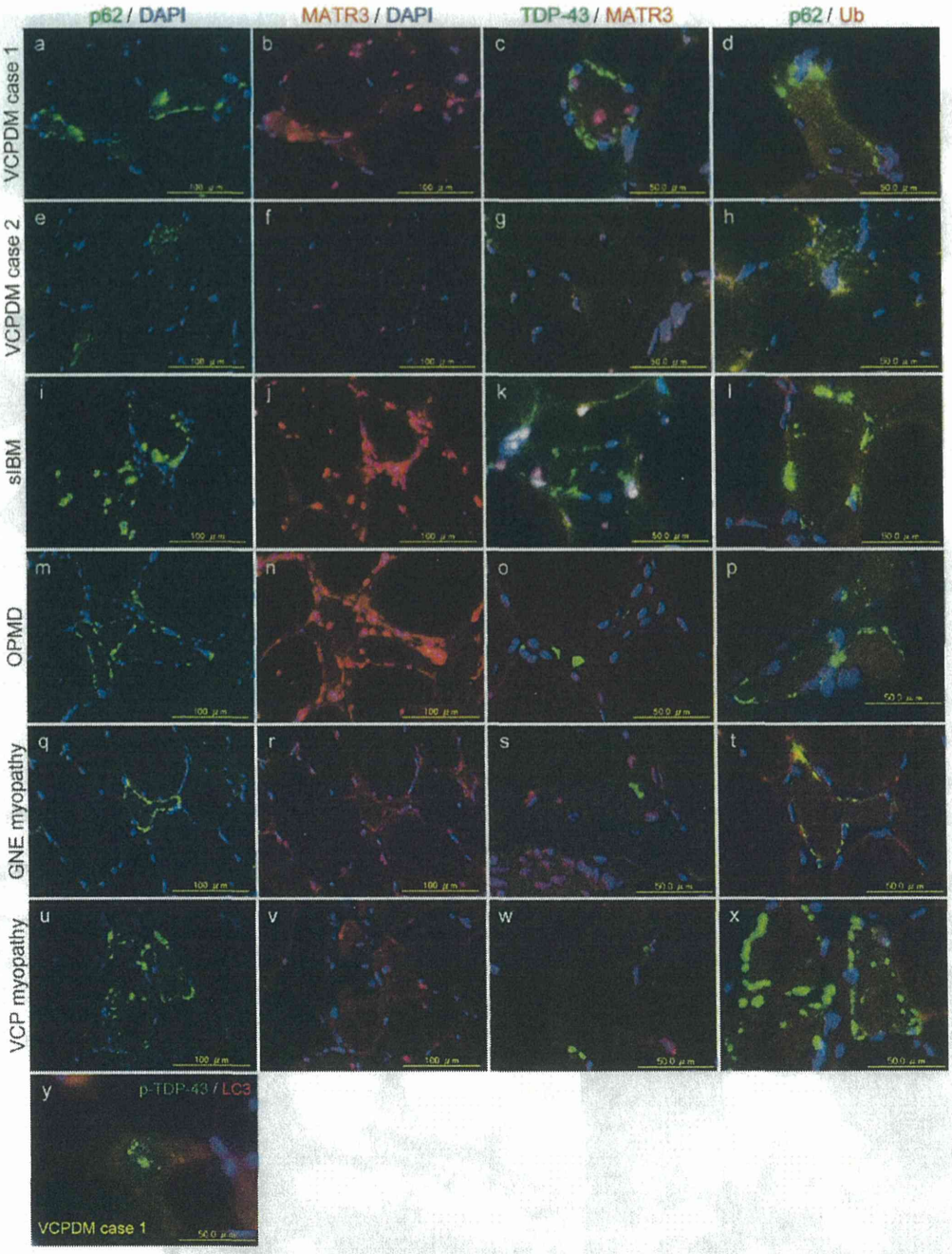

Figure 2. Immunofluorescence studies for proteins related to myopathies with rimmed vacuoles. Immunofluorescence study of p62 (green; **a**, **e**, **i**, **m**, **q**, **u**) and MATR3 (red; **b**, **f**, **j**, **n**, **r**, **v**) in identical specimens from VCPDM case 1 (**a**, **b**), case 2 (**e**, **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.

Acknowledgements

We would like to thank Ms Oka and all members of Department of Neurology, Kumamoto University Hospital for the technical help with muscle biopsies and clinical data collection. This research was supported by a Grant-in-Aid for Scientific Research (C), the Ministry of Education, Culture, Sports, Science and Technology of Japan; Grants-in-Aid from the Research on Intractable Diseases, Ministry of Health, Labor and Welfare of Japan; and Grants-in-Aid from the Research Committee for Applying Health and Technology of Ministry of Health, Welfare and Labor, Japan.

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.

S. Yamashita*
A. Mori*
Y. Nishida†
R. Kurisaki‡
N. Tawara*
T. Nishikami*
Y. Misumi*
H. Ueyama†
S. Imamura†
Y. Higuchi§
A. Hashiguchi§
I. Higuchi§
S. Morishita¶
J. Yoshimura¶
M. Uchino*
H. Takashima§
S. Tsuji**
Y. Ando*

**Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, †Department of Neurology, National Hospital Organization, Kumamoto Saishunsou National Hospital, ‡Department of Neurology, National Hospital Organization, Kumamoto Minami National Hospital, Kumamoto, §Department of Neurology and Geriatrics, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, and ¶Department of Computational Biology, Graduate School of Frontier Sciences and **Department of Neurology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan*

References

1 Udd B. Distal myopathies–new genetic entities expand diagnostic challenge. *Neuromuscul Disord* 2012; **22**: 5–12
2 Feit H, Silbergleit A, Schneider LB, Gutierrez JA, Fitoussi RP, Reyes C, Rouleau GA, Brais B, Jackson CE, Beckmann JS, Seboun E. Vocal cord and pharyngeal weakness with autosomal dominant distal myopathy: clinical description and gene localization to 5q31. *Am J Hum Genet* 1998; **63**: 1732–42
3 Senderek J, Garvey SM, Krieger M, Guergueltcheva V, Urtizberea A, Roos A, Elbracht M, Stendel C, Tournev I, Mihailova V, Feit H, Tramonte J, Hedera P, Crooks K, Bergmann C, Rudnik-Schoneborn S, Zerres K, Lochmuller H, Seboun E, Weis J, Beckmann JS, Hauser MA, Jackson CE. Autosomal-dominant distal myopathy associated with a recurrent missense mutation in the gene encoding the nuclear matrix protein, matrin 3. *Am J Hum Genet* 2009; **84**: 511–18
4 Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 2009; **25**: 1754–60
5 Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 2009; **25**: 2078–9
6 Dubourg O, Wanschitz J, Maisonobe T, Behin A, Allenbach Y, Herson S, Benveniste O. Diagnostic value of markers of muscle degeneration in sporadic inclusion body myositis. *Acta Myol* 2011; **30**: 103–8
7 Weihl CC, Pestronk A. Sporadic inclusion body myositis: possible pathogenesis inferred from biomarkers. *Curr Opin Neurol* 2010; **23**: 482–8
8 D'Agostino C, Nogalska A, Engel WK, Askanas V. In sporadic inclusion body myositis muscle fibres TDP-43-positive inclusions are less frequent and robust than p62 inclusions, and are not associated with paired helical filaments. *Neuropathol Appl Neurobiol* 2011; **37**: 315–20
9 Nogalska A, Terracciano C, D'Agostino C, King Engel W, Askanas V. p62/SQSTM1 is overexpressed and prominently accumulated in inclusions of sporadic inclusion-body myositis muscle fibers, and can help differentiating it from polymyositis and dermatomyositis. *Acta Neuropathol* 2009; **118**: 407–13
10 Tasca G, Ricci E, Monforte M, Laschena F, Ottaviani P, Rodolico C, Barca E, Silvestri G, Iannaccone E, Mirabella M, Broccolini A. Muscle imaging findings in GNE myopathy. *J Neurol* 2012; **259**: 1358–65
11 Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM, McCluskey LF, Miller BL, Masliah E, Mackenzie IR, Feldman H, Feiden W, Kretzschmar HA, Trojanowski JQ, Lee VM. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 2006; **314**: 130–3
12 Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, Mann D, Tsuchiya K, Yoshida M, Hashizume Y, Oda T. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun* 2006; **351**: 602–11
13 Weihl CC, Temiz P, Miller SE, Watts G, Smith C, Forman M, Hanson PI, Kimonis V, Pestronk A. TDP-43 accumulation in inclusion body myopathy muscle suggests a common pathogenic mechanism with frontotemporal dementia. *J Neurol Neurosurg Psychiatry* 2008; **79**: 1186–9
14 Salajegheh M, Pinkus JL, Taylor JP, Amato AA, Nazareno R, Baloh RH, Greenberg SA. Sarcoplasmic redistribution of nuclear TDP-43 in inclusion body myositis. *Muscle Nerve* 2009; **40**: 19–31
15 Olive M, Janue A, Moreno D, Gamez J, Torrejon-Escribano B, Ferrer I. TAR DNA-Binding protein 43 accumulation in protein aggregate myopathies. *J Neuropathol Exp Neurol* 2009; **68**: 262–73

16 Kusters B, van Hoeve BJ, Schelhaas HJ, Ter Laak H, van Engelen BG, Lammens M. TDP-43 accumulation is common in myopathies with rimmed vacuoles. *Acta Neuropathol* 2009; **117**: 209–11
17 Yamashita S, Kimura E, Tawara N, Sakaguchi H, Nakama T, Maeda Y, Hirano T, Uchino M, Ando Y. Optineurin is potentially associated with TDP-43 and involved in the pathogenesis of inclusion body myositis. *Neuropathol Appl Neurobiol* 2013; **39**: 406–16
18 Neumann M, Mackenzie IR, Cairns NJ, Boyer PJ, Markesbery WR, Smith CD, Taylor JP, Kretzschmar HA, Kimonis VE, Forman MS. TDP-43 in the ubiquitin pathology of frontotemporal dementia with VCP gene mutations. *J Neuropathol Exp Neurol* 2007; **66**: 152–7
19 Johnson JO, Pioro EP, Boehringer A, Chia R, Feit H, Renton AE, Pliner HA, Abramzon Y, Marangi G, Winborn BJ, Gibbs JR, Nalls MA, Morgan S, Shoai M, Hardy J, Pittman A, Orrell RW, Malaspina A, Sidle KC, Fratta P, Harms MB, Baloh RH, Pestronk A, Weihl CC, Rogaeva E, Zinman L, Drory VE, Borghero G, Mora G, Calvo A, Rothstein JD, Drepper C, Sendtner M, Singleton AB, Taylor JP, Cookson MR, Restagno G, Sabatelli M, Bowser R, Chio A, Traynor BJ. Mutations in the Matrin 3 gene cause familial amyotrophic lateral sclerosis. *Nat Neurosci* 2014; **17**: 664–6
20 Salton M, Lerenthal Y, Wang SY, Chen DJ, Shiloh Y. Involvement of Matrin 3 and SFPQ/NONO in the DNA damage response. *Cell Cycle* 2010; **9**: 1568–76

Received 17 April 2014
Accepted after revision 13 August 2014
Published online Article Accepted on 4 September 2014

ANNALS of Clinical and Translational Neurology
Open Access
ANA American Neurological Association

RESEARCH ARTICLES

Variants associated with Gaucher disease in multiple system atrophy

Jun Mitsui[1], Takashi Matsukawa[1], Hidenao Sasaki[2], Ichiro Yabe[2], Masaaki Matsushima[2], Alexandra Dürr[3], Alexis Brice[3], Hiroshi Takashima[4], Akio Kikuchi[5], Masashi Aoki[5], Hiroyuki Ishiura[1], Tsutomu Yasuda[1], Hidetoshi Date[1], Budrul Ahsan[1], Atsushi Iwata[1], Jun Goto[1], Yaeko Ichikawa[1], Yasuo Nakahara[1], Yoshio Momose[1], Yuji Takahashi[1], Kenju Hara[6], Akiyoshi Kakita[7], Mitsunori Yamada[7,8], Hitoshi Takahashi[7], Osamu Onodera[6], Masatoyo Nishizawa[6], Hirohisa Watanabe[9], Mizuki Ito[9], Gen Sobue[9], Kinya Ishikawa[10], Hidehiro Mizusawa[10], Kazuaki Kanai[11], Takamichi Hattori[11], Satoshi Kuwabara[11], Kimihito Arai[12], Shigeru Koyano[13], Yoshiyuki Kuroiwa[14], Kazuko Hasegawa[15], Tatsuhiko Yuasa[16], Kenichi Yasui[17], Kenji Nakashima[17], Hijiri Ito[18], Yuishin Izumi[19], Ryuji Kaji[19], Takeo Kato[20], Susumu Kusunoki[21], Yasushi Osaki[22], Masahiro Horiuchi[23], Tomoyoshi Kondo[24], Shigeo Murayama[25], Nobutaka Hattori[26], Mitsutoshi Yamamoto[27], Miho Murata[28], Wataru Satake[29], Tatsushi Toda[29], Alessandro Filla[30], Thomas Klockgether[31], Ullrich Wüllner[31], Garth Nicholson[32], Sid Gilman[33], Caroline M. Tanner[34], Walter A. Kukull[35], Mathew B. Stern[36], Virginia M.-Y. Lee[37], John Q. Trojanowski[37], Eliezer Masliah[38], Phillip A. Low[39], Paola Sandroni[39], Laurie J. Ozelius[40], Tatiana Foroud[41] & Shoji Tsuji[1]

[1]Department of Neurology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan
[2]Department of Neurology, Hokkaido University Graduate School of Medicine, Sapporo, Japan
[3]AP-HP, Hôpital de la Salpêtrière, Département de Génétique et Cytogénétique, Inserm, U 1127, Cnrs, UMR 7225, 3- Sorbonne Université, UPMC Univ Paris 06, UM 75, ICM, F-75013, Paris, France
[4]Department of Neurology and Geriatrics, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan
[5]Department of Neurology, Tohoku University School of Medicine, Sendai, Japan
[6]Department of Neurology, Brain Research Institute, Niigata University, Niigata, Japan
[7]Department of Pathology, Brain Research Institute, Niigata University, Niigata, Japan
[8]Department of Clinical Research, Saigata Medical Center, National Hospital Organization, Niigata, Japan
[9]Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Japan
[10]Department of Neurology and Neurological Science, Graduate School of Medical and Dental Science, Tokyo Medical and Dental University, Tokyo, Japan
[11]Department of Neurology, Chiba University School of Medicine, Chiba, Japan
[12]Division of Neurology, National Hospital Organization, Chiba East Hospital, Chiba, Japan
[13]Department of Clinical Neurology and Stroke Medicine, Graduate School of Medicine, Yokohama City University, Yokohama, Japan
[14]Department of Neurology, Teikyo University School of Medicine University Hospital, Mizonokuchi, Kawasaki, Japan
[15]Division of Neurology, National Hospital Organization, Sagamihara National Hospital, Sagamihara, Japan
[16]Department of Neurology, Kamagaya-Chiba Medical Center for Intractable Neurological Disease, Kamagaya General Hospital, Chiba, Japan
[17]Division of Neurology, Department of Brain and Neurosciences, Faculty of Medicine, Tottori University, Yonago, Japan
[18]Department of Neurology, Mifukai Vihara Hananosato Hospital, Hiroshima, Japan
[19]Department of Clinical Neuroscience, Institute of Health Biosciences, University of Tokushima Graduate School, Tokushima, Japan
[20]Departments of Neurology, Hematology, Metabolism, Endocrinology, and Diabetology, Faculty of Medicine, Yamagata University, Yamagata, Japan
[21]Department of Neurology, Kinki University School of Medicine, Osaka, Japan
[22]Department of Geriatrics, Cardiology and Neurology, Kochi Medical School, Nankoku, Japan
[23]Division of Neurology, Department of Internal Medicine, St. Marianna University School of Medicine, Kawasaki, Japan
[24]Department of Neurology, Wakayama Medical University, Wakayama, Japan
[25]Department of Neuropathology and the Brain Bank for Aging Research, Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology, Tokyo, Japan
[26]Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan
[27]Department of Neurology, Kagawa Prefectural Central Hospital, Takamatsu, Japan
[28]Department of Neurology, National Center Hospital of Neurology and Psychiatry, Tokyo, Japan
[29]Division of Neurology/Molecular Brain Science, Kobe University Graduate School of Medicine, Kobe, Japan
[30]Department of Neurological Sciences, University Federico II, Naples, Italy
[31]Department of Neurology, University of Bonn and German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany
[32]Concord Hospital, University of Sydney at the Australian and New Zealand Army Corps (ANZAC) Research Institute, Sydney, Australia
[33]Department of Neurology, University of Michigan, Ann Arbor, Michigan
[34]Parkinson's Disease Research Education and Clinical Center, San Francisco Veteran's Affairs Medical Center, San Francisco, California

[35]Department of Epidemiology, University of Washington School of Public Health, Seattle, Washington
[36]Parkinson's Disease and Movement Disorders Center, Department of Neurology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania
[37]Institute on Aging, Udall Parkinson's Research Center, Center for Neurodegenerative Disease Research and the Department of Pathology and Laboratory Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania
[38]Department of Neurosciences, University of California San Diego, San Diego, California
[39]Department of Neurology, Mayo Clinic, Rochester, Minnesota
[40]Departments of Genetics and Genomic Sciences and Neurology, Icahn School of Medicine at Mount Sinai, New York, New York
[41]Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, Indiana

Correspondence
Shoji Tsuji, 7-3-1 Hongo, Bunkyo, Tokyo 113-8655, Japan. Tel: +81-3-5800-8972; Fax: +81-3-5800-6548; E-mail: tsuji@m.u-tokyo.ac.jp

Funding Information
This study was supported in part by KAKENHI (Grant-in-Aid for Scientific Research on Innovative Areas [22129001 and 22129002]) to S. T.; the Global COE Program from the Ministry of Education, Culture, Sports, Science and Technology of Japan, a Grant-in-Aid (H23-Jitsuyoka [Nanbyo]-Ippan-004, and "the Research Committee for Ataxic Diseases" of the Research on Measures for Intractable Diseases) from the Ministry of Health, Welfare and Labour, Japan to S. T.; the French Agency for Research (ANR-09-MNPS-032-01/R09148DS) and the Programme Hospitalier de Recherche Clinique (AOM03059/R05129DD) to A. D. and A. B.; the Deutsche Forschungsgemeinschaft (Wu 184-6) the Deutsche Parkinson Vereinigung to U. W.; National Institutes of Health grant (NS-53488) to J. Q. T.; and National Institutes of Health grant (P01NS044233) to P. A. L.

Received: 12 January 2015; Revised: 28 January 2015; Accepted: 28 January 2015

doi: 10.1002/acn3.185

Abstract

Objective: Glucocerebrosidase gene (*GBA*) variants that cause Gaucher disease are associated with Parkinson disease (PD) and dementia with Lewy bodies (DLB). To investigate the role of *GBA* variants in multiple system atrophy (MSA), we analyzed *GBA* variants in a large case–control series. **Methods**: We sequenced coding regions and flanking splice sites of *GBA* in 969 MSA patients (574 Japanese, 223 European, and 172 North American) and 1509 control subjects (900 Japanese, 315 European, and 294 North American). We focused solely on Gaucher-disease-causing *GBA* variants. **Results**: In the Japanese series, we found nine carriers among the MSA patients (1.65%) and eight carriers among the control subjects (0.89%). In the European series, we found three carriers among the MSA patients (1.35%) and two carriers among the control subjects (0.63%). In the North American series, we found five carriers among the MSA patients (2.91%) and one carrier among the control subjects (0.34%). Subjecting each series to a Mantel–Haenszel analysis yielded a pooled odds ratio (OR) of 2.44 (95% confidence interval [CI], 1.14–5.21) and a *P*-value of 0.029 without evidence of significant heterogeneity. Logistic regression analysis yielded similar results, with an adjusted OR of 2.43 (95% CI 1.15–5.37) and a *P*-value of 0.022. Subtype analysis showed that Gaucher-disease-causing *GBA* variants are significantly associated with MSA cerebellar subtype (MSA-C) patients ($P = 7.3 \times 10^{-3}$). **Interpretation**: The findings indicate that, as in PD and DLB, Gaucher-disease-causing *GBA* variants are associated with MSA.

Introduction

Multiple system atrophy (MSA) is a progressive neurodegenerative disease characterized clinically by autonomic failure in addition to various combinations of parkinsonism, cerebellar ataxia, and pyramidal dysfunction.[1] The cardinal neuropathological hallmark is argyrophilic filamentous glial cytoplasmic inclusions (GCIs)[2,3] in which fibrillar aggregates of α–synuclein in oligodendrocytes are the major components.[4,5]

Until recently, MSA had been defined as a nongenetic disorder, but then several multiplex families with the disease were described, triggering extensive fruitful searches for susceptibility genes in case–control association studies.[6,7] Subsequently, we identified a homozygous mutation and compound heterozygous mutations of *COQ2* in two multiplex families with MSA.[8] We also found a common variant (V393A) and multiple rare variants in *COQ2*, all of which lead to functional impairments in the *COQ2* gene product that increase the risk of developing sporadic MSA.

V393A was observed exclusively in the Japanese population, and the carrier frequency of V393A was significantly higher in Japanese MSA patients (9.1%) than in Japanese controls (3.3–4.4%) with odds ratios of 2.1–3.0. These findings suggest that impaired COQ2 activity, which would be predicted to impair the mitochondrial respiratory chain and increase vulnerability to oxidative stress, causes susceptibility to MSA. As the association was observed only in a small proportion of MSA patients, the pathogenic mechanisms underlying MSA largely remain unknown.

In contrast to the GCIs found in MSA, neuronal inclusions containing α–synuclein, termed Lewy bodies (LBs), are observed neuropathologically in Parkinson disease (PD) and dementia with LBs (DLB).[9] Abnormal fibrillar α–synuclein aggregation in LBs and GCIs is the common feature of PD/DLB and MSA, respectively, which collectively form a subset of neurodegenerative disorders referred to as the "α–synucleinopathies".[10] Intriguingly, a link between PD and MSA was reported recently based on postmortem brain examinations of familial PD patients with *SNCA* mutations (G51D and A53E) showing α–synuclein pathology characterized by neuronal and oligodendroglial inclusions similar to GCIs.[11,12] Moreover, the risk of parkinsonism among first-degree relatives is significantly higher in MSA patients than in control subjects,[13,14] and coincidence of PD and MSA within the same pedigrees has been reported.[15] Taken together, these findings raise the possibility that MSA and PD share some genetic basis.

Glucocerebrosidase (*GBA*) genetic variants that have been proven to be pathogenic for Gaucher disease (GD variants) are strongly associated with PD and DLB.[16–18] Initial focus upon GD variants came from a report of several families of patients with GD in which obligate or confirmed carriers frequently developed parkinsonism.[19] As then, many studies have shown that GD variants are associated with PD and DLB.[16–18]

In this study, we analyzed *GBA* in a large series of sporadic MSA patients and control subjects to investigate the role of GD variants in the pathogenesis of MSA.

Methods

Patients with sporadic MSA and control subjects

All patients with sporadic MSA and healthy control subjects described in the previous reports,[8,16] and additional participants (210 MSA patients and 380 control subjects in the Japanese series) were enrolled in this study. Written informed consent was obtained from all participants in accordance with research protocols that were approved by institutional review boards at participating centers. The diagnoses of possible and probable MSA were made based upon current consensus criteria.[1] A total of 574 patients with sporadic MSA and 900 control subjects were included in the Japanese series, 223 patients and 315 control subjects in the European series and 172 patients and 294 control subjects in the North American series (persons of European or Hispanic descent living in North America). Ancestry was determined by self-report on a multiple-choice questionnaire. The North American series comprised 160 persons of European descent and 12 persons of Hispanic descent in patients and 284 persons of European descent and 10 persons of Hispanic descent in control subjects. Among the 218 MSA patients in the Japanese series where information on family history was available (159 with MSA cerebellar subtype [MSA-C], 53 with MSA of the parkinsonism subtype [MSA-P] and six with MSA of undefined subtype), there were 22 MSA patients (11 MSA-C, 10 MSA-P, and one undefined subtype) who also had relatives with the clinical diagnosis of PD. Among these relatives with PD, genomic DNAs were available from five (in five families) who had siblings with MSA. Sporadic MSA patients and control subjects were recruited without reference to the presence of family history for parkinsonism. Demographic characteristics are shown in Table 1. In the European series, the male to female ratio was significantly higher in cases than that in controls ($P = 0.0012$). In the Japanese series, the mean age at examination was significantly older in cases than that in controls ($P < 0.0001$). The MSA-P to MSA-C ratio was significantly higher in the North American series than that in the Japanese series and the European series ($P < 0.0001$ and $P < 0.0001$, respectively).

Multiplex families with MSA

Independent of the sporadic case–control series, six previously described multiplex Japanese families (1–4, 8, and 12: same families with those in a previous report)[8] were also screened for *GBA* variants. Autopsy findings of two affected members in Family 1 and III-6 in Family 8 confirmed the diagnosis of MSA. As previously reported, affected members in Family 1 carried the homozygous M128V-V393A variant in *COQ2* and those in Family 12 carried the compound heterozygous R387X/V393A variants in *COQ2*.[8]

Nucleotide sequence analysis of GBA

Polymerase chain reaction products were subjected to direct nucleotide sequence analysis of coding sequences and flanking splice sites of *GBA* with a DNA analyzer, 3730*xl* (Life Technologies, Carlsbad, CA). Three primer pairs were designed selectively to amplify *GBA* but not its pseudogene, as previously described.[16,20] Analysis of sequence traces was achieved using Variant Reporter v1.1

Table 1. Demographic data of participants.

	Japanese series		European series		North American series	
	MSA patients	Control subjects	MSA patients	Control subjects	MSA patients	Control subjects
N	574	900	223	315	172	294
Age at onset	58.7, 8.7	NA	55.4, 8.3	NA	58.4, 9.5	NA
Age at examination	62.8, 8.3	51.1, 16.7	59.2, 8.0	58.9, 6.1	ND	65.2, 9.0
Sex (male/female)	306/268	434/466	138/85	150/165	103/69	156/138
Clinical subtype (MSA-C/MSA-P/Undefined)	403/141/30	NA	191/22/10	NA	52/107/13	NA

Values are presented as means and standard deviations. NA, not applicable; ND, not described; MSA-C, multiple system atrophy cerebellar subtype; MSA-P, multiple system atrophy parkinsonism subtype.

(Life Technologies) and by manual inspection of electropherograms. All sequencing analysis was performed at the Medical Genome Center, The University of Tokyo Hospital.

Nomenclature of GBA variants

Amino acid numbering of *GBA* variants followed conventional nomenclature, which considers the first amino acid after the signal peptide (the first amino acid of the mature GBA protein) as amino acid 1.[21]

Gaucher-disease-causing GBA variants

We referred to the Human Gene Mutation Database (HGMD) Professional 2014.1 (BIOBASE, Beverly, MA) for information about *GBA* variants. The variants that were categorized as "disease-causing mutations" for GD in HGMD are hereinafter termed GD variants. In this study, we focused solely on GD variants, which included 256 missense variants, 19 splicing variants, 28 small deletions, 15 small insertions, four small indels, four gross deletions (defined as more than 20 base-pairs), one gross insertion, and 18 complex rearrangements.

Statistical analysis

Results are presented as means and standard deviations. We used Student's *t*-test to determine whether the mean age at disease onset between carriers and noncarriers of the GD variants were significantly different. We used Fisher's exact test and multiple logistic regression analysis to calculate the significance of differences in allele frequencies. We used multiple logistic regression analysis to compute odds ratios and corresponding 95% confidence intervals (CIs). We calculated pooled odds ratios based on a fixed-effects model (Mantel–Haenszel method) and a multiple logistic regression model. The heterogeneity across odds ratios was assessed with Cochran *Q* statistic, Breslow-Day test, and I^2 statistics. All statistical tests were two-sided, and we used a *P*-value of less than 0.05 to indicate statistical significance. Our statistical analysis utilized StatsDirect version 2.7.8 (StatsDirect, Cheshire, England) and R verison 2.15.3 (http://r-project.org/).

Results

We identified 20 nonsynonymous single-nucleotide substitutions and one complex multiple-nucleotide substitutions (L444P-A456P-V460V or Rec*Nci*I) in our sporadic case–control series. Among these 21 variants, nine were known GD variants (R120W, G202R, F213I, N370S, G377S, D409H, L444P, L444R, and Rec*Nci*I) (Table 2), that is, all nine have been proven to be pathogenic for GD, which were identified only in cases with clinical features of GD and decreased GBA activities.[22–29] The other 12 variants have not been reported to be pathogenic for GD, and include I(-20)V, P55L, Q57R, L67Q, R163Q, I204M, E326K, T334I, F347L, T369M, T410R, and I489V (Table S1). Of note, it has been shown that activities of mutant GBA with E326K are slightly to moderately decreased. The consensus is that E326K is not sufficient to cause GD, and a functional polymorphism.[30] With these considerations, we did not include E326K as GD-causing mutations.

In the Japanese series, we found nine carriers of GD variants among 574 MSA patients (1.65%) and eight carriers among 900 control subjects (0.89%). In the European series, we found three carriers among 223 MSA patients (1.35%) and two carriers among 315 control subjects (0.63%). In the North American series, we found five carriers among 172 MSA patients (2.91%) and one carrier among 294 control subjects (0.34%). Combining all series, we identified 17 carriers among 969 MSA patients (1.75%) as GD variant carriers, and we found GD variant carriers in 11 of the 1509 control subjects (0.73%). Among carriers of GD variants, two MSA patients (one MSA-C from the European series and one MSA-P from the North American series) carried homozygous N370S variants, whereas none of the control subjects had two alleles with GD variants. The ages at onset of the

Table 2. Gaucher-disease-causing *GBA* variants in sporadic MSA patients and control subjects in each series.

	Japanese series		European series		North American series	
Genotypes	MSA patients (n = 574)	Control subjects (n = 900)	MSA patients (n = 223)	Control subjects (n = 315)	MSA patients (n = 172)	Control subjects (n = 294)
R120W/NM	1	0	0	0	0	0
G202R/NM	1	0	0	0	0	0
F213I/NM	2	0	0	0	0	1
N370S/NM	0	0	1	2	2	0
N370S/N370S	0	0	1	0	1	0
G377S/NM	0	0	0	0	1	0
D409H/NM	0	1	0	0	0	0
L444P/NM	4	2	1	0	1	0
L444R/NM	1	0	0	0	0	0
Rec*Nci*I/NM	0	5	0	0	0	0
Total	9/574 (1.65%)	8/900 (0.89%)	3/223 (1.35%)	2/315 (0.63%)	5/172 (2.91%)	1/294 (0.34%)
Odds ratio (95% confidence interval)	1.78 (0.68–4.76)		2.13 (0.35–16.3)		8.77 (1.34–168.8)	
Fisher's exact test	P = 0.32		P = 0.65		P = 0.028	

Logistic regression analysis	MSA patients (n = 969)	Control subjects (n = 1509)
Carrier frequency of Gaucher-disease-causing *GBA* variants	17/969 (1.75%)	11/1509 (0.73%)
Odds ratio adjusted for each series (95% confidence interval)	2.43 (1.15–5.37), P = 0.022	
Odds ratio unadjusted for each series (95% confidence interval)	2.43 (1.15–5.37), P = 0.022	

GBA, Glucocerebrosidase; MSA, multiple system atrophy; NM, nonmutated allele.

MSA-C and MSA-P patients were 43 and 69, respectively. The available medical records of the two cases made no mention of GD or relevant clinical signs.

Although the carrier frequencies of GD variants were higher in MSA patients than in control subjects within the three series, the difference was significant only in the North American series (P = 0.028). A Mantel–Haenszel procedure of each series yielded a pooled odds ratio (OR) of 2.44 (95% CI 1.14–5.21) and a P-value of 0.029 (Fig. 1). The heterogeneity of OR of each series from the Mantel–Haenszel analysis was not significant (Cochran Q = 1.80, P = 0.41; Breslow-Day = 1.99, P = 0.37; I^2 = 0%). A multiple logistic regression model employing GD variants in each series yielded an OR adjusted for each series of 2.43 (95% CI = 1.15–5.37, P = 0.022), and an unadjusted OR of 2.43 (95% CI = 1.15–5.37, P = 0.022) (Table 2). Taken together, these data indicate that there is no effect modification by the series.

We then analyzed the clinical presentations of the 17 MSA patients carrying GD variants. The ages (in years) at symptom onset in these patients (58.1 ± 8.2) did not differ significantly (P = 0.93) from those in noncarriers (57.9 ± 8.9). The male to female ratio of these patients (8–9) did not differ significantly (P = 0.47) from those of noncarriers (540 to 412). The clinical phenotypes of these 17 MSA patients included 14 MSA-C and three MSA-P subjects (Table 3). The carrier frequency of MSA-C patients was 2.17% (14 in 646) and that of MSA-P patients was 1.11% (3 in 270). None of the 53 patients with undefined subtypes carried GD variants. Given that 11 in 1509 control subjects (0.73%) carried such variants, GD variants were significantly associated with MSA-C (adjusted OR, 2.99 [95% CI 1.35–6.79], $P = 7.3 \times 10^{-3}$). In the MSA-C group, a Mantel–Haenszel procedure of each series yielded a pooled OR of 3.00 (95% CI 1.37–6.59) with a P-value of 6.3×10^{-3} (Fig. S1). The heterogeneity of OR of each series from the Mantel–Haenszel analysis was not significant (Cochran Q = 2.82, P = 0.24; Breslow-Day = 3.48, P = 0.18; I^2 = 29%). Although the carrier frequency is also higher in MSA-P patients than in control subjects, the association of GD variants with MSA-P is inconclusive (adjusted OR, 1.54 [95% CI 0.34–5.04], P = 0.51).

Interestingly, we occasionally see siblings or other family members of the patients with MSA who are affected with PD. Among the five sib-pairs with sporadic MSA and PD, where genomic DNA samples are available (Families P2, P29, P30, P31, and P32 in Fig. 2), one sib-pair (Family P29) share the same heterozygous GD mutation, G202R. In another sib-pair (Family P2), one PD patient discordantly had a heterozygous GD variant (Rec*Nci*I). *SNCA* single-nucleotide substitutions and multiplications were not present in the affected members of these pedigrees (data not shown).

Independent of the above analyses, we additionally analyzed affected members in six multiplex families with MSA reported previously.[8] Of the six multiplex MSA

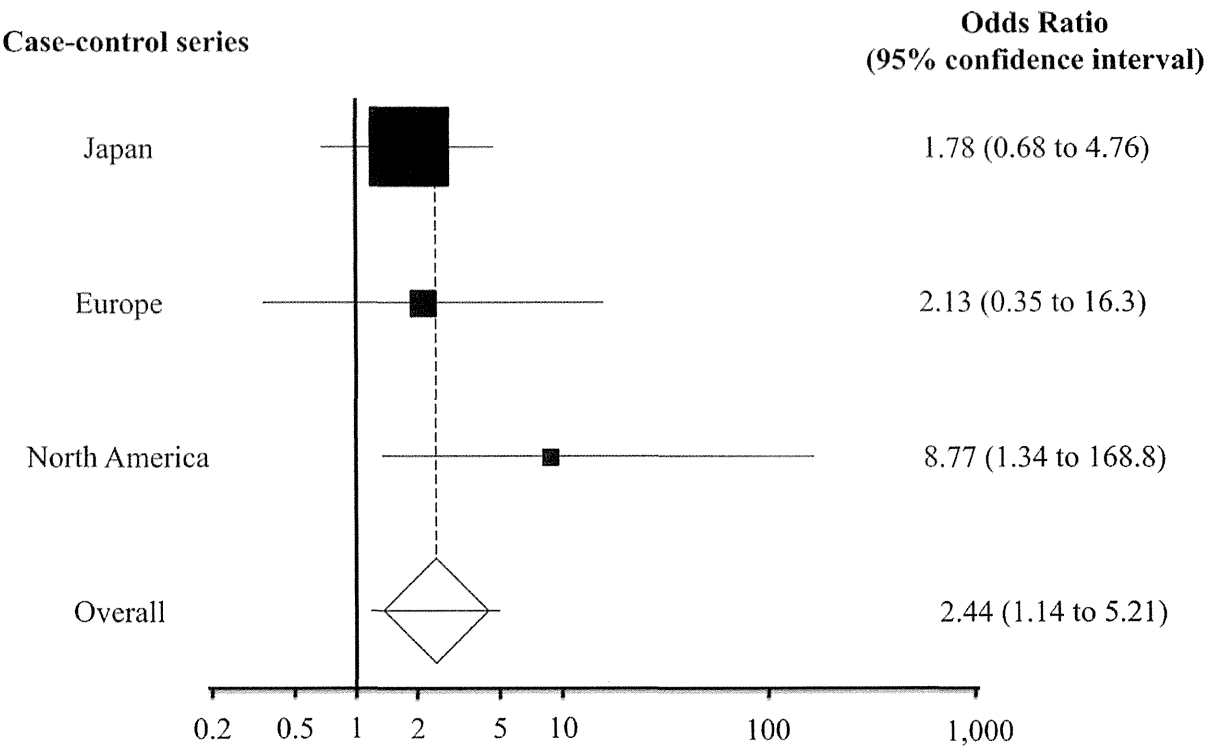

Figure 1. Odds ratios for GD-causing *GBA* variants among MSA patients, as compared with controls, at each case–control series and overall. Shown are the combined estimates (on a $\log_{10}$ scale) of the odds ratios for carrying GD variants. The odds ratio estimate is marked with a solid black square. The lines represent the 95% confidence interval of odds ratio estimate. The size of the square represents the weight that the corresponding series exerts in the Mantel–Haenszel analysis. Confidence intervals of pooled odds ratios are displayed as a horizontal line through the diamond. The heterogeneity of odds ratio of each series from the Mantel–Haenszel analysis was not significant (Cochran $Q = 1.80$, $P = 0.41$; Breslow-Day = 1.99, $P = 0.37$; $I^2 = 0\%$), indicating that there is no effect modification by the series (population). GBA, Glucocerebrosidase; MSA, multiple system atrophy; GD, Gaucher disease.

Table 3. Gaucher-disease-causing *GBA* variants in combined series in each clinical subtype.

	Cases			Controls
Genotypes	MSA-C patients (n = 646)	MSA-P patients (n = 270)	Undefined subtypes patients (n = 53)	Control subjects (n = 1509)
R120W/NM	1	0	0	0
G202R/NM	1	0	0	0
F213I/NM	2	0	0	1
N370S/NM	2	1	0	0
N370S/N370S	1	1	0	2
G377S/NM	1	0	0	0
D409H/NM	0	0	0	1
L444P/NM	5	1	0	2
L444R/NM	1	0	0	0
Rec*Nci*I/NM	0	0	0	5
Total	14/646 (2.17%)	3/270 (1.11%)	0/53 (0.00%)	11/1509 (0.73%)
Adjusted odds ratio (95% confidence interval)	2.99 (1.35–6.79)	1.54 (0.34–5.04)	NA	NA
Fisher's exact test	$P = 7.3 \times 10^{-3}$	$P = 0.51$	NA	NA

GBA, Glucocerebrosidase; MSA-C, multiple system atrophy of the cerebellar type; MSA-P, multiple system atrophy parkinsonism subtype; NM, nonmutated allele; NA, not applicable.

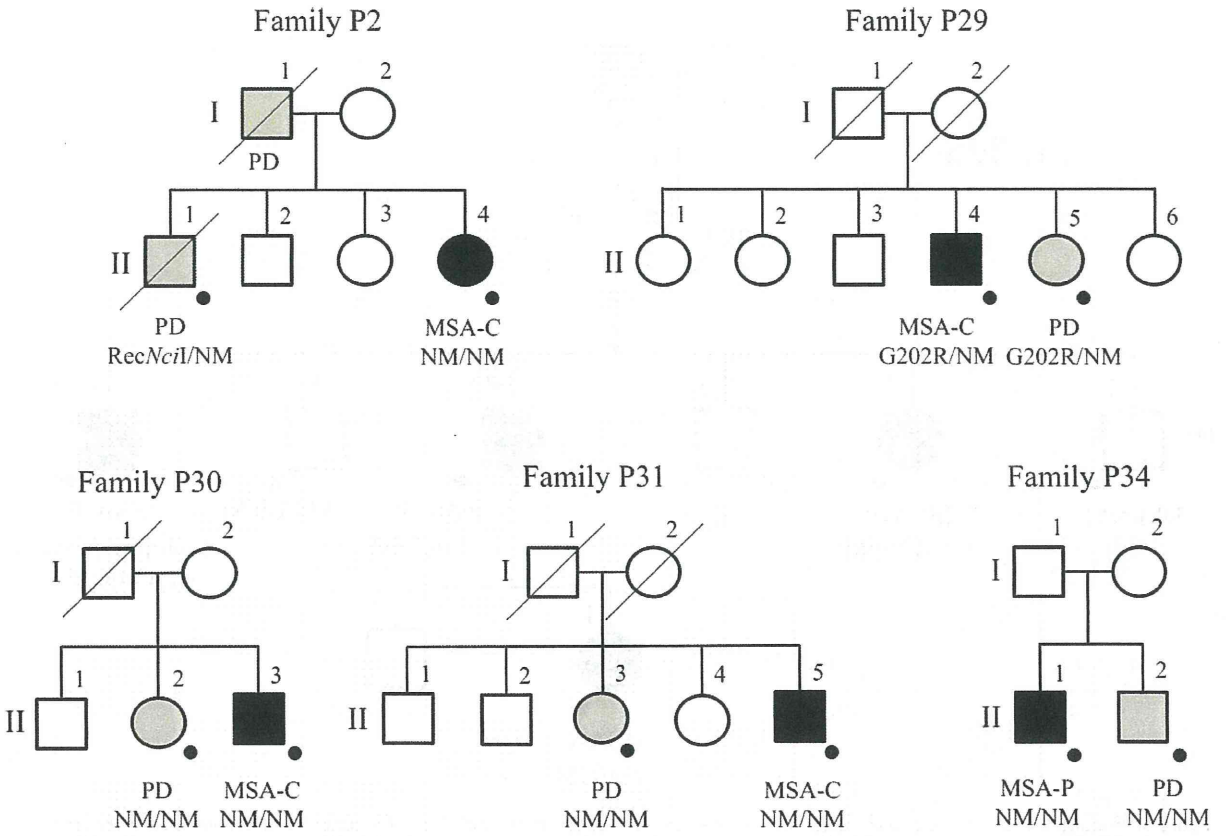

Figure 2. Identification of Gaucher-disease-causing *GBA* variants in sib-pairs with coincidence of MSA and PD. Squares represent men; circles, women; black symbols, individuals with MSA; gray symbols, individuals with PD; open symbols, unaffected individuals; dots, genomic DNAs available. GBA, Glucocerebrosidase; MSA-C, multiple system atrophy of the cerebellar type; MSA-P, multiple system atrophy with predominant parkinsonism; PD, Parkinson disease; NM, nonmutated allele.

families, we found that three of the four patients with MSA in Family 8 had GD variants, whereas we did not observe GD variants in other families. In Family 8, three patients with MSA-P (III-2, III-4, and III-6) including one (III-6) with autopsy-proven MSA (definite MSA) had the same heterozygous GD variant (L444R) (Fig. 3), whereas the other patient with MSA-C (IV-1) did not carry the variant. In addition, one unaffected sibling (III-5) carried the variant, whereas the other unaffected sibling (III-1) did not carry the variant. Thus, although co-segregation of the GD variant with MSA was not complete in this family, the observation may also support some association of GD variants with MSA.

Discussion

In this study, we demonstrated that GD variants are associated with MSA, raising a possibility that MSA, PD, and DLB partly share genetic risk factors. The carrier frequency of GD variants in MSA is 1.75% in the combined Japanese, European, and North American series. This is in a striking contrast to a much higher carrier frequency of ~7% in PD, suggesting that impact of GD variants as a risk factor for MSA is weaker compared to that for PD.[17]

We focused solely on GD variants that have been reported to be pathogenic mutations for GD, and this makes it difficult to interpret the pathogenicity of other rare variants that have not been reported to cause GD. As a considerable number of rare variants with unknown significance were identified in this study, functional analysis of each mutant GBA would be required to determine whether they are functionally neutral variants or potentially pathogenic for GD.

Diagnosis of MSA was made according to the current consensus criteria in this study. Although there is an inherent risk for clinical misdiagnosis, which would limits the interpretations of our findings, it should also be noted that to evaluate associations of rare variants with diseases as encountered in this study, substantially large sample sizes are required to accomplish a sufficient detection power. Nonetheless, it is noteworthy that GD variants were significantly associated with MSA-C, given that the clinical

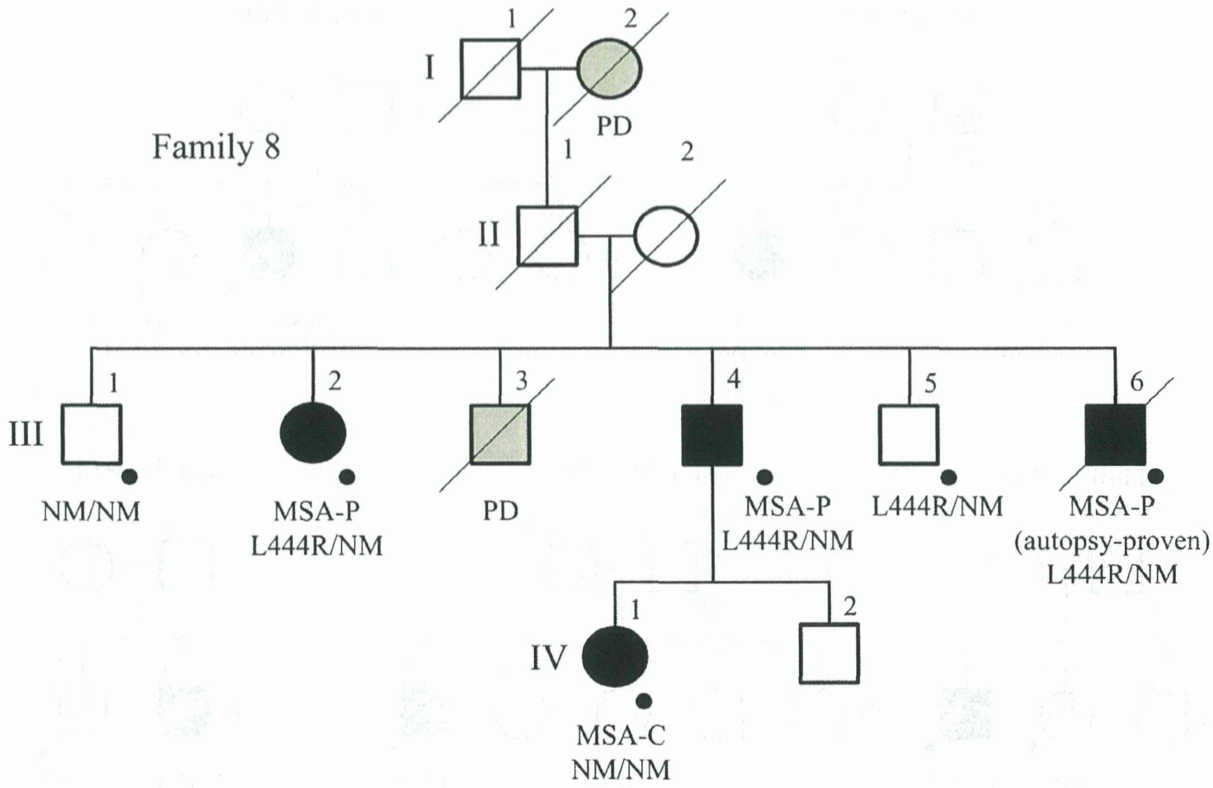

Figure 3. Identification of Gaucher-disease-causing *GBA* variants in a multiplex family with MSA. The diagnosis of definite MSA in III-6 in Family 8 was confirmed by autopsy findings. Squares represent men; circles, women; black symbols, individuals with MSA; gray symbols, individuals with PD; open symbols, unaffected individuals; dots, genomic DNAs available. GBA, Glucocerebrosidase; MSA-P, multiple system atrophy with predominant parkinsonism; PD, Parkinson disease; NM, nonmutated allele.

presentation of MSA-C is distinct from that of PD and inclusion of patients with PD in the MSA-C group is unlikely.[1] Although the carrier frequency is also higher in MSA-P patients than in control subjects, the association of GD variants with MSA-P is inconclusive. Given their limited sample size (n = 270) in this study, analysis of a larger case series will be needed to answer the question of whether GD variants are also associated with MSA-P.

In the five sib-pairs with coincidence of MSA and PD and the six multiplex families with MSA, it is noteworthy that, despite the low frequency of carriers of the GD variants in the Japanese controls (0.89%), GD variants were identified, and shared by affected siblings in Family P29 (one sibling with MSA-C and one with PD) and Family 8 (three siblings with MSA-P). None of the affected individuals (either MSA or PD) carried deleterious variants in *COQ2* (data not shown). Although the number of families is limited and cosegregation is not complete in Family 8, these observations may support the increased risk of GD variants in developing MSA, leading to familial clustering.

In our previous study, we showed that the carrier frequency of V393A in *COQ2* was significantly higher in Japanese MSA patients (9.1%) than in Japanese controls (3.3–4.4%) with odds ratios of 2.1–3.0.[8] It is remarkable that among the nine MSA patients carrying GD variants in the Japanese series, two MSA-C patients had GD variants (one F213I and one L444P) and V393A variants in *COQ2* simultaneously, both in heterozygous states (data not shown). Their ages at onset were both 54 years, which is slightly younger than the average age at onset of 58.7 years in the Japanese MSA patients. As the number of patients carrying both alleles is limited, the biological relevance of the combination of *GBA* and *COQ2* variants in the pathogenesis of MSA remains to be determined.

Previous studies have shown no significant associations of GD variants with MSA,[31–34] perhaps because of smaller sample sizes, and perhaps because these studies analyzed only specific variants (L444P and/or N370S). The concordant trend of increased risk of GD variants for developing MSA in each of the three series utilized in this study (Japanese, European, and North American) strengthens our conclusion and makes a bias of stratification unlikely.

Since discovery of the genetic association between *GBA* and PD, the biological relationships between mutant GBA

and α–synuclein have been investigated intensively. Use of induced pluripotent stem cells from PD patients with GD variants revealed that GBA activity was reduced, glucosylceramide and α–synuclein levels were increased, and both autophagy and ubiquitin-proteasome pathways were defective in derived dopaminergic neurons.[35] Moreover, a postmortem human brain study showed that, even in sporadic PD patients without GD variants, GBA activity was selectively reduced in the early stages of PD within regions containing increased α–synuclein levels and limited LB formation.[36] While future replication studies on larger case–control series would be needed to verify the association of *GBA* mutations with MSA, studies on the biochemical effects associated with mutant *GBA* may contribute to better understanding the mechanisms underlying development of MSA as well as those underlying PD, and aid in developing novel therapeutic measures for this intractable disease.

Acknowledgments

This study was supported in part by KAKENHI (Grant-in-Aid for Scientific Research on Innovative Areas [22129001 and 22129002]) to S. T.; the Global COE Program from the Ministry of Education, Culture, Sports, Science and Technology of Japan, a Grant-in-Aid (H23-Jitsuyoka [Nanbyo]-Ippan-004, and "the Research Committee for Ataxic Diseases" of the Research on Measures for Intractable Diseases) from the Ministry of Health, Welfare and Labour, Japan to S. T.; the French Agency for Research (ANR-09-MNPS-032-01/R09148DS) and the Programme Hospitalier de Recherche Clinique (AOM03059/R05129DD) to A. D. and A. B.; the Deutsche Forschungsgemeinschaft (Wu 184-6) the Deutsche Parkinson Vereinigung to U. W.; National Institutes of Health grant (NS-53488) to J. Q. T.; and National Institutes of Health grant (P01NS044233) to P. A. L. We thank Mio Takeyama, Keiko Hirayama, and Zhenghong Wu for their support in the laboratory experiments and Cecilia Marelli for her clinical input to this study. We also thank the DNA and Cell Bank of CRicm for technical assistance.

Conflict of Interest

None declared.

References

1. Gilman S, Wenning GK, Low PA, et al. Second consensus statement on the diagnosis of multiple system atrophy. Neurology 2008;71:670–676.
2. Papp MI, Kahn JE, Lantos PL. Glial cytoplasmic inclusions in the CNS of patients with multiple system atrophy (striatonigral degeneration, olivopontocerebellar atrophy and Shy-Drager syndrome). J Neurol Sci 1989;94:79–100.
3. Nakazato Y, Yamazaki H, Hirato J, et al. Oligodendroglial microtubular tangles in olivopontocerebellar atrophy. J Neuropathol Exp Neurol 1990;49:521–530.
4. Tu PH, Galvin JE, Baba M, et al. Glial cytoplasmic inclusions in white matter oligodendrocytes of multiple system atrophy brains contain insoluble alpha-synuclein. Ann Neurol 1998;44:415–422.
5. Wakabayashi K, Yoshimoto M, Tsuji S, Takahashi H. Alpha-synuclein immunoreactivity in glial cytoplasmic inclusions in multiple system atrophy. Neurosci Lett 1998;249:180–182.
6. Al-Chalabi A, Durr A, Wood NW, et al. Genetic variants of the alpha-synuclein gene SNCA are associated with multiple system atrophy. PLoS One 2009;4:e7114.
7. Scholz SW, Houlden H, Schulte C, et al. SNCA variants are associated with increased risk for multiple system atrophy. Ann Neurol 2009;65:610–614.
8. The Multiple-System Atrophy Research Collaboration. Mutations in COQ2 in familial and sporadic multiple-system atrophy. N Engl J Med 2013;369:233–244.
9. Spillantini MG, Schmidt ML, Lee VM, et al. Alpha-synuclein in Lewy bodies. Nature 1997;388:839–840.
10. Goedert M, Spillantini MG. Lewy body diseases and multiple system atrophy as alpha-synucleinopathies. Mol Psychiatry 1998;3:462–465.
11. Kiely AP, Asi YT, Kara E, et al. α-Synucleinopathy associated with G51D SNCA mutation: a link between Parkinson's disease and multiple system atrophy? Acta Neuropathol 2013;125:753–769.
12. Pasanen P, Myllykangas L, Siitonen M, et al. A novel α-synuclein mutation A53E associated with atypical multiple system atrophy and Parkinson's disease-type pathology. Neurobiol Aging 2014;35:2180.e1–2180.e5.
13. Vidal JS, Vidailhet M, Derkinderen P, et al. Familial aggregation in atypical Parkinson's disease: a case control study in multiple system atrophy and progressive supranuclear palsy. J Neurol 2010;257:1388–1393.
14. Nee LE, Gomez MR, Dambrosia J, et al. Environmental-occupational risk factors and familial associations in multiple system atrophy: a preliminary investigation. Clin Auton Res 1991;1:9–13.
15. Fujioka S, Ogaki K, Tacik PM, et al. Update on novel familial forms of Parkinson's disease and multiple system atrophy. Parkinsonism Relat Disord 2014;20(Suppl 1):S29–S34.
16. Mitsui J, Mizuta I, Toyoda A, et al. Mutations for Gaucher disease confer high susceptibility to Parkinson disease. Arch Neurol 2009;66:571–576.
17. Sidransky E, Nalls MA, Aasly JO, et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. N Engl J Med 2009;361:1651–1661.

18. Nalls MA, Duran R, Lopez G, et al. A multicenter study of glucocerebrosidase mutations in dementia with Lewy bodies. JAMA Neurol 2013;70:727–735.
19. Goker-Alpan O, Schiffmann R, LaMarca ME, et al. Parkinsonism among Gaucher disease carriers. J Med Genet 2004;41:937–940.
20. Koprivica V, Stone DL, Park JK, et al. Analysis and classification of 304 mutant alleles in patients with type 1 and type 3 Gaucher disease. Am J Hum Genet 2000;66:1777–1786.
21. Tsuji S, Choudary PV, Martin BM, et al. Nucleotide sequence of cDNA containing the complete coding sequence for human lysosomal glucocerebrosidase. J Biol Chem 1986;261:50–53.
22. Stone DL, Carey WF, Christodoulou J, et al. Type 2 Gaucher disease: the collodion baby phenotype revisited. Arch Dis Child Fetal Neonatal Ed 2000;82: F163–F166.
23. Kim MJ, Suh JT, Lee HJ, et al. Simultaneous detection of Gaucher's disease and renal involvement of non-Hodgkin's lymphoma: the first Asian case report and a review of literature. Ann Clin Lab Sci 2012;42:293–301.
24. He GS, Grace ME, Grabowski GA. Gaucher disease: four rare alleles encoding F213I, P289L, T323I, and R463C in type 1 variants. Hum Mutat 1992;1:423–427.
25. Cormand B, Grinberg D, Gort L, et al. Two new mild homozygous mutations in Gaucher disease patients: clinical signs and biochemical analyses. Am J Med Genet 1997;70:437–443.
26. Amaral O, Marcão A, Sá Miranda M, et al. Gaucher disease: expression and characterization of mild and severe acid beta-glucosidase mutations in Portuguese type 1 patients. Eur J Hum Genet 2000;8:95–102.
27. Michelakakis H, Dimitriou E, Van Weely S, et al. Characterization of glucocerebrosidase in Greek Gaucher disease patients: mutation analysis and biochemical studies. J Inherit Metab Dis 1995;18:609–615.
28. Walley AJ, Barth ML, Ellis I, et al. Gaucher's disease in the United Kingdom: screening non-Jewish patients for the two common mutations. J Med Genet 1993;30:280–283.
29. Uchiyama A, Tomatsu S, Kondo N, et al. New Gaucher disease mutations in exon 10: a novel L444R mutation produces a new NciI site the same as L444P. Hum Mol Genet 1994;3:1183–1184.
30. Horowitz M, Pasmanik-Chor M, Ron I, Kolodny EH. The enigma of the E326K mutation in acid β-glucocerebrosidase. Mol Genet Metab 2011;104:35–38.
31. Segarane B, Li A, Paudel R, et al. Glucocerebrosidase mutations in 108 neuropathologically confirmed cases of multiple system atrophy. Neurology 2009;72:1185–1186.
32. Jamrozik Z, Lugowska A, Slawek J, Kwiecinski H. Glucocerebrosidase mutations p.L444P and p.N370S are not associated with multisystem atrophy, progressive supranuclear palsy and corticobasal degeneration in Polish patients. J Neurol 2010;257:459–460.
33. Sun QY, Guo JF, Han WW, et al. Genetic association study of glucocerebrosidase gene L444P mutation in essential tremor and multiple system atrophy in mainland China. J Clin Neurosci 2013;20:217–219.
34. Srulijes K, Hauser AK, Guella I, et al. No association of GBA mutations and multiple system atrophy. Eur J Neurol 2013;20:e61–e62.
35. Schöndorf DC, Aureli M, McAllister FE, et al. iPSC-derived neurons from GBA1-associated Parkinson's disease patients show autophagic defects and impaired calcium homeostasis. Nat Commun 2014;5:4028.
36. Murphy KE, Gysbers AM, Abbott SK, et al. Reduced glucocerebrosidase is associated with increased α-synuclein in sporadic Parkinson's disease. Brain 2014;137:834–848.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Odds ratios for Gaucher-disease-causing *GBA* variants among MSA-C patients, as compared with controls, at each case–control series and overall. Shown are the combined estimates (on a $\log_{10}$ scale) of the odds ratios for carrying GD variants among MSA-C patients. The odds ratio estimate is marked with a solid black square. The lines represent the 95% confidence interval of odds ratio estimate. The size of the square represents the weight that the corresponding series exerts in the Mantel–Haenszel analysis. Confidence intervals of pooled odds ratios are displayed as a horizontal line through the diamond. The heterogeneity of odds ratio of each series from the Mantel–Haenszel analysis was not significant (Cochran $Q = 2.82$, $P = 0.24$; Breslow-Day = 3.48, $P = 0.18$; $I^2 = 29\%$), indicating that there is no effect modification by the series (population).

Table S1. Nonsynonymous variants in *GBA* that have not been reported to be causative for Gaucher disease.

Bioinformatics Advance Access published November 8, 2013

Genetics and population analysis

Rapid detection of expanded short tandem repeats in personal genomics using hybrid sequencing

Koichiro Doi[1], Taku Monjo[1,2], Pham H. Hoang[1,2], Jun Yoshimura[1], Hideaki Yurino[1], Jun Mitsui[3], Hiroyuki Ishiura[3], Yuji takahashi[3], Yaeko Ichikawa[3], Jun Goto[3], Shoji Tsuji[3] and Shinichi Morishita[1,*]

[1]Department of Computational Biology, Graduate School of Frontier Sciences, The University of Tokyo, Chiba 277-8562, Japan

[2]Department of Information and Communication Engineering, Faculty of Engineering , The University of Tokyo, Tokyo 113-8656, Japan

[3]Department of Neurology, Graduate School of Medicine, The University of Tokyo, Tokyo 113-8655, Japan

Associate Editor: Dr. Jeffrey Barrett

ABSTRACT

Motivation: Long expansions of short tandem repeats (STRs); i.e., DNA repeats of 2-6 nucleotides, are associated with some genetic diseases. Cost-efficient high throughput sequencing can quickly produce billions of short reads that would be useful for uncovering disease-associated STRs. However, enumerating STRs in short reads remains largely unexplored because of the difficulty in elucidating STRs much longer than 100 bp, the typical length of short reads.

Results: We propose *ab initio* procedures for sensing and locating long STRs promptly by utilizing the frequency distribution of all STRs and paired-end read information. We validated the reproducibility of this method using biological replicates and used it to locate an STR associated with a brain disease (SCA31). Subsequently, we sequenced this STR site in eleven SCA31 samples using SMRT™ sequencing (Pacific Biosciences), determined 2.3-3.1 kb sequences at nucleotide resolution, and revealed that (TGGAA)- and (TAAAATAGAA)-repeat expansions determined the instability of the repeat expansions associated with SCA31. Our method could also identify common STRs, (AAAG)- and (AAAAG)-repeat expansions, which are remarkably expanded at four positions in an SCA31 sample. This is the first proposed method for rapidly finding disease-associated long STRs in personal genomes using hybrid sequencing of short and long reads.

Availability: Our TRhist software is available at http://trhist.gi.k.u-tokyo.ac.jp/.

Contact: moris@cb.k.u-tokyo.ac.jp

Supplementary Information: Supplementary data are available at *Bioinformatics* online.

1 INTRODUCTION

Many genetic disorders are caused by or associated with short tandem repeats (STRs), repetitive elements of 2-6 nucleotides. Regarding the mechanism underlying the phenomenon of repeat expansion, unusual structural features of repeat-containing regions that affect cellular replication, repair, and recombination are thought to induce frequent replication slippage, thereby expanding repeats (Mirkin, 2007). STRs have been found in a variety of genomic regions. Huntington's disease is associated with expansion of the triplet repeat $(CAG)_n$ (polyglutamine runs in proteins) in the coding region of huntingtin (The Huntington's Disease Collaborative Research Group, 1993), where $n < 28$ in normal samples, n = 28-35 in intermediate cases, n = 36-40 in reduced penetrance, and $n > 40$ in full penetrance (Walker, 2007). Spinal and bulbar muscular atrophy is also associated with (CAG) repeats in one exon (La Spada, *et al.*, 1991).

In addition to exons, STRs have been observed in a variety of genomic regions such as untranslated regions (UTRs), introns, and promoters. Fragile-X syndrome is associated with (CGG) repeat in the 5'-UTR (Kremer, *et al.*, 1991; Sherman, *et al.*, 1985; Verkerk, *et al.*, 1991), and myotonic dystrophy type 1 (DM1) with (CTG) repeat in the 3'-UTR (Brook, *et al.*, 1992; Mahadevan, *et al.*, 1992). In introns, spinocerebellar ataxia type 10 (SCA10) is associated with (ATTCT) repeat (Matsuura, *et al.*, 2000), myotonic dystrophy type 2 (DM2) with (CCTG) repeat (Liquori, *et al.*, 2001), amyotrophic lateral sclerosis / frontotemporal dementia (ALS/FTD) with (GGGGCC) repeat (DeJesus-Hernandez, *et al.*, 2011; Orr, 2011; Renton, *et al.*, 2011), and SCA36 with (GGCCTG) repeat (Kobayashi, *et al.*, 2011). Consequently, whole genome sequencing capable of observing non-exonic regions is required to characterize STRs peculiar to a personal genome.

Several expanded repeats in RNA, such as CUG, CCUG, CAG, CGG, AUUCU, and UGGAA, are associated with hereditary diseases and are known to accumulate in nuclear RNA foci in which several proteins are sequestrated in the process of foci formation (for a review see Wojciechowska and Krzyzosiak, 2011). These RNA foci are thought to have a negative effect on host cells, leading to disorders in cellular pathways (Wojciechowska and Krzyzosiak, 2011).