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**Clinical, pathological, and genetic mutation analysis of sporadic inclusion body
myositis in Japanese**

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Abstract

Previous studies have identified several genetic loci associated with development of familial inclusion body myopathy. However, there have been few genetic analyses of sporadic inclusion body myositis (sIBM). In order to explore the molecular basis of sIBM and to investigate genotype-phenotype correlations, we performed a clinicopathological analysis of 21 sIBM patients and screened for mutations in the *Desmin*, *GNE*, *MYHC2A*, *VCP*, and *ZASP* genes. All coding exons of the 5 genes were sequenced directly. Definite IBM was confirmed in 14 cases, probable IBM in 3 cases, and possible IBM in 4 cases. No cases showed missense mutations in the *Desmin*, *GNE*, or *VCP* genes. Three patients carried the missense mutation c.2542T>C (p.V805A) in the *MYHC2A* gene; immunohistochemical staining for MYHC isoforms in these 3 cases showed atrophy or loss of muscle fibers expressing MYHC IIa or IIx. One patient harbored the missense mutation c.1719G>A (p.V566M) in the *ZASP* gene; immunohistochemical studies of Z-band associated proteins revealed Z-band abnormalities. Both of the novel heterogeneous mutations were located in highly evolutionarily conserved domains of their respective genes. Cumulatively, these findings have expanded our understanding of the molecular background of sIBM. However, we advocate further clinicopathology and investigation of additional candidate genes in a larger cohort.

Keywords: inclusion body myositis, desmin, UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase (GNE), myosin heavy chain IIa (MYHC2A), valosin-containing protein (VCP), Z-band alternatively spliced PDZ-motif containing protein (ZASP)

Introduction

Inclusion body myositis (IBM), the most common muscle disease affecting adults >50 years old, is increasing in frequency in Japan [1]. Clinical features of IBM include adult-onset weakness of proximal or distal muscle and normal or slightly elevated serum creatine kinase (CK) levels. Electromyography (EMG) typically reveals myogenic abnormalities or a mixed pattern of both myogenic and neurogenic changes. Rimmed vacuoles have been proposed as one of the main histopathological features necessary for diagnosing IBM [2]. However, rimmed vacuoles are not specific to IBM. For instance, they may be observed in patients with inclusion body myopathy with Paget disease and frontotemporal dementia (IBMPFD), as well as in those with myofibrillar myopathy (MFM). In general, clinical features of IBM may be diverse and show some overlap with other conditions—in particular, hereditary inclusion body myopathy (hIBM). Because of this, it can be difficult to distinguish between IBM and the overlapping diseases using clinical features and pathological findings alone. Therefore, genetic studies and immunochemical staining may provide important differentiation clues.

Although the etiopathogenesis of IBM is enigmatic, it is thought to involve a complex interaction of degeneration, genetic factors, and environmental influences. The few studies that have investigated the genetic background of IBM have failed to provide conclusive results [3, 4]. However, research on the genetics of other inclusion body myopathies may provide important clues about IBM. For instance, a single gene mutation is thought to be responsible for hIBM, IBMPFD, and zaspopathy (Table 1). Molecular genetic studies have identified 3 loci for hIBM: IBM1, IBM2, and IBM3 [5, 6, 7]. Over the past 10 years, there has been an accumulation of evidence suggesting

that IBM1, or desmin-related myopathy (DRM; OMIM #601419), is caused by mutations in the *Desmin* gene on chromosome 2q35 [5]. DRM, a form of myofibrillar myopathy (MFM), is characterized by a combination of skeletal muscle weakness and cardiomyopathy [5, 8]. Most of the known mutations of the *Desmin* gene are autosomal dominant, but some are autosomal recessive and a significant number of these mutations are *de novo* [8]. Autosomal recessive IBM2 (OMIM #600737) is characterized by weakness of distal muscles in the lower limbs but a sparing of quadriceps muscles, and is induced by mutations in the UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase (*GNE*) gene mapped to chromosome 9p13.3 [6]. Mutations in this gene are also known to be responsible for distal myopathy with rimmed vacuole (DMRV), also named Nonaka myopathy (OMIM #605820), a condition that has been reported predominantly in the Japanese population and has an autosomal recessive mode of inheritance. The shared genotype and similar phenotypes of DMRV and IBM2 indicates that they are allelic disorders [9, 10]. An autosomal dominant type of IBM3 (OMIM #605637), characterized by joint contractures, external ophthalmoplegia, and proximal muscle weakness, results from mutations in the gene encoding myosin heavy chain IIa (*MYHC2A* or *MYH2*); this is located on chromosome 17p13.1 [7,11,12]. A recent report that myopathy patients from 3 families carried compound heterozygous mutations in the *MYHC2A* gene implies that this disorder is inherited via autosomal recessive transmission [12].

In addition to the 3 types of hIBM, another form of the overlapping diseases is IBMPFD (OMIM #167320). This rare autosomal dominant disorder is caused by missense mutations in the gene encoding valosin-containing protein (VCP); this is located on chromosome 9p13.3 [13, 14]. Zaspopathy (OMIM #609452), another subtype

of MFM, manifests as a late-onset, autosomal dominant distal myopathy with or without cardiomyopathy. The gene responsible for this disorder is located on chromosome 10q23.2 and codes for Z-band alternatively spliced PDZ-motif containing protein (ZASP) [15, 16]. Cumulatively, the familial inclusion body myopathies show heterogeneous patterns of inheritance and may present even when there is no family history as a result of recessive inheritance, an incomplete penetrance of the dominant inheritance, or *de novo* mutations. This explains reports of non-familial, sporadic cases with mutations in these genes [8, 16, 17].

Immunohistochemistry may be efficient in diagnosis as it reflects the protein deposit in cells or tissue. A definite diagnosis of IBM should include an immunohistochemical investigation of amyloid deposits, CD8⁺ T cell expression, and the upregulation of MHC-I expression [2]. On the other hand, immunostaining for MYHC isoforms may demonstrate the expression of different MYHC isoforms in different muscle in a single section, providing an indication of MYHC protein function [18]. Additionally, immunostaining for Z-band associated protein has been reported as the most reliable immunocytochemical markers for the diagnosis of MFM [19]. Characterization of these causative genes has begun to provide valuable insights into the pathogenesis of related diseases and a need for candidate gene studies for sIBM. However, the extent to which these genes play a role in the pathogenesis of sIBM is poorly understood. Thus, we developed the current study to explore the molecular basis of sIBM and to investigate genotype-phenotype correlations that may be helpful for diagnosis. Specifically, we analyzed the clinical features of 21 patients with sIBM and screened for mutations in the *Desmin*, *GNE*, *MYHC2A*, *VCP*, and *ZASP* genes.

Patients and methods

Patients

We retrospectively reviewed a total of 645 cases with available muscle pathology from April 1994 to March 2010 in the Department of Neurology of the Hokkaido University Graduate School of Medicine. Of these, we recruited 21 patients (12 males, 9 females) who met the following inclusion criteria: (1) symptom duration >6 months; (2) age at onset >30 years; (3) slowly progressive muscle weakness and wasting; (4) myofiber necrosis and regeneration in muscle biopsy; (5) endomysial mononuclear cell infiltration in muscle biopsy; and (6) vacuolated muscle fibers in muscle biopsy [2]. We excluded 2 cases from the same family, who had distal myopathy with rimmed vacuoles caused by *GNE* mutations [9]. Cases of glycogenosis and lipid storage myopathy were also excluded from the study. There was no known familial relationship between any of the included patients and the 80 ethnically matched control subjects. We performed a detailed retrospective review of the clinical and pathological findings. This study was approved by the Medical Ethics Committee of Hokkaido University Graduate School of Medicine.

Histochemistry

Flash-frozen muscle specimens were stored in liquid nitrogen until use. Samples from all patients underwent a battery of conventional pathological studies, including hematoxylin-eosin (HE), modified Gomori trichrome (mGT), nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-tr), adenosine triphosphatase (ATPase; pre-incubation at pH 4.3, 4.6, and 10), non-specific esterase, and alkaline phosphatase.

Cytochrome c oxidase (COX) studies could only be performed in 16 of the 21 patients, as a result of insufficient quantities of muscle tissue.

Immunohistochemical studies

To diagnose IBM, we performed immunostaining for the β -amyloid peptide precursor (β APP) N-terminus and C-terminus in all 21 patients. Immunostaining for CD8⁺ T cells, ubiquitin, and MHC class I expression could only be performed in 16 of 21 patients as a result of insufficient muscle specimen samples.

Muscle biopsy specimens (1 from the quadriceps and 2 from the biceps brachii) were obtained from 3 patients with an *MYHC2A* variant. To detect MYHC isoform protein expression in different muscle, double immunostaining for MYHC isoform were performed. The MYHC isoform immunostaining used slow myosin monoclonal antibody against slow type I (ATPase type 1) fibers (WB-MHCs, Abcam, Cambridge, MA, USA), and myosin A4.74 (Enzo life sciences, Farmingdale, NY, USA), which is a monoclonal antibody against fast type IIa (ATPase type 2A) fibers [18]. The immunostaining was performed using the official protocol of Nichirei Biosciences (Tokyo, Japan).

To diagnose zaspopathy, quadriceps biopsy specimens, obtained from the patient who was known to have a ZASP mutation (age at biopsy = 63 years), were used for immunohistochemical staining of Z-band associated proteins. The following antigens were used in the immunoreactions: myotilin, desmin, α -B crystalline (α -BC), dystrophin C-terminus, neural cell adhesion molecule (NCAM), cell division cycle 2 (CDC2) kinase, ubiquitin, and β APP N-terminus and C-terminus [19].

Molecular genetic studies

We extracted genomic DNA from frozen muscle or blood using the standard protocols associated with the Viogene Blood & Tissue Genomic DNA Extraction Miniprep System (Gentaur, Belgium). We used PCR to amplify the 9 coding exons of the *Desmin* gene, 13 coding exons of the *GNE* gene, 38 coding exons of the *MYHC2A* gene, 17 coding exons of the *VCP* gene, and 16 coding exons of the *ZASP* gene, as well as the flanking non-coding regions of the above; this was accomplished using the primers and methods reported previously [6, 7, 20, 21]. Sequencing was performed using a BigDye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems, Carlsbad, CA, USA). Sequencing products were purified by BigDye X Terminator (Applied Biosystems) and analyzed on an ABI3130 genetic analyzer with sequencing analyzer software (Applied Biosystems, Foster City, California, USA).

Statistical analysis

All data were analyzed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). We used a X^2 test to evaluate differences in relative risk (RR) and the frequencies of the variant of the *MYHC2A* gene between patients and controls. The risk of developing the disease was expressed as RR and associated 95% confidence intervals (CIs). All statistical tests were two-sided, and significance was defined as $P < 0.05$.

Results

Clinicopathological features of sIBM patients

Characteristics of the 21 sIBM patients are listed in Tables 2 and 3. No patients were aware of any family history of sIBM. The mean age at onset was 58.0 ± 11.4 years

(range: 35-73 years), and the mean age at biopsy was 63.4 ± 10.7 years (range: 37-75 years). All patients presented with slowly progressive weakness. The majority of patients ($n = 13$, 61.9%) experienced proximal dominant weakness, while only 2 patients were distal dominant (Patients 7 and 14); all remaining patients ($n = 6$) had equal weakness of proximal and distal muscles. None of the patients exhibited cardiomyopathy.

Biopsy samples were collected from the quadriceps in 12 patients (57.1%) and from the biceps in 9 patients (42.9%; Table 2). Serum CK levels were elevated in 18 of the 20 patients (90%) in whom they could be measured (mean \pm SD, 626.3 ± 390.3 U/L; normal range: 40-265 U/L); only Patients 2 and 7 had normal serum CK levels. Of the 16 patients in whom serum aldolase could be measured, high levels were found in 10 (62.5%) and normal levels were found in 6 (mean \pm SD, 6.43 ± 4.32 U/L; normal range: 2.0-5.8 U/L). Thirteen patients underwent needle electromyographic (EMG) studies, which revealed myogenic motor unit potentials (MUPs) in 8 patients (61.5%) and a mixed pattern in 2 patients (Patients 4 and 13). Neurogenic MUPs were found for the 3 remaining patients, one of whom (Patient 1) had diabetes mellitus.

Inflammatory infiltration and rimmed vacuoles were observed in the light microscopy analyses of samples taken from all patients (100%). Myopathic features, such as variation in fiber diameters, necrosis, and regeneration of muscle fibers, were also present in samples from all patients (Table 3). β APP immunostaining was positive in 20 patients (95.2%); immunoreactivity to CD8⁺ T cell and upregulation of MHC class I expression appeared in 16 patients (100%); COX-negative fibers were observed in 15 patients (93.8%); and positive staining of ubiquitin was present in 14 patients (87.5%). According to the criteria of Needham and Mastaglia [2], 14 patients were diagnosed as

having definite IBM, 3 patients were diagnosed as having probable IBM, and 4 patients were diagnosed as having possible IBM (Table 3).

Mutation analysis

None of the 21 patients harbored missense mutations in the *Desmin*, *GNE*, or *VCP* genes. Direct sequencing of the *MYHC2A* gene showed a heterozygous base substitution in exon 19 (c.2542T>C) in 3 patients (2 males and 1 female; Patients 3, 10, 12) (Fig. 1a), which resulted in an amino acid substitution of alanine for valine at codon 805 (p.V805A). The p.V805A variant was only present in 1 of 160 chromosomes obtained from our 80 control individuals. Thus, the frequency of this allele was significantly higher in the sIBM patients than in the controls (7.1% vs. 0.6%, respectively; $p < 0.01$). Further, the p.V805A variant of the *MYHC2A* gene was associated with a significantly increased risk of sIBM (RR = 12.2; 95% CI = 1.24-120.8).

ZASP gene sequencing analysis revealed a heterozygous base change, G to A, in exon 15 (NM_001080114.1(LDB3_v2): c.1719G>A) (Fig. 1b) in 1 patient (Patient 7). The change in the first base of codon 566 resulted in an amino acid conversion of valine to methionine (NM_001080114.1(LDB3_v2): p.V566M). The codon was numbered on transcript variant 2 of the *Homo sapiens LDB3* gene at http://www.ncbi.nlm.nih.gov/nuccore/NM_001080114.1 (the *ZASP* gene is also known as *LDB3* because it encodes the LIM domain-binding 3). This mutation was not present in any of the 160 control chromosomes.

Neither the c.1719G>A heterozygous point mutation in the *ZASP* gene nor the c.2542T>C base substitution in the *MYHC2A* gene have been reported in any databases as known mutations or single nucleotide polymorphisms (SNPs;

<http://www.ncbi.nlm.nih.gov/SNP>, <http://snp.ims.u-tokyo.ac.jp/search.html>). Further, both the novel p.V805A *MYHC2A* variant and p.V566M *ZASP* mutation are located in the evolutionarily conserved domains of their respective genes (Fig. 1c, 1d; <http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Clinical-Genetic correlations

MYHC2A p.V805A variant

The 3 patients with the p.V805A missense mutation in the *MYHC2A* gene (Patients 3, 10, and 12) were 52, 67, and 66 years old at onset, and underwent biopsy was 59, 72, and 68 years old, respectively. All 3 patients had slowly progressive weakness dominant in the proximal extremities and displayed a clinical phenotype resembling that of sIBM. None of the patients reported joint contractures or external ophthalmoplegia, a family history of sIBM, or consanguineous marriage. Serum CK levels were elevated in all patients at the time of biopsy (1,096, 576, and 483 U/L, respectively). Serum aldolase was elevated only in 1 patient (9.4 U/L).

EMG studies revealed myopathic patterns only in 1 patient. Muscle histopathology showed rimmed vacuoles with CD8⁺ T cell infiltration, and ATPase histochemistry revealed selective atrophy or loss of type 2 fibers. All 3 patients were classified as “definite IBM” according to the criteria [2]. Immunohistochemical staining for MYHC isoforms showed that 2 patients had atrophy of muscle fibers expressing MYHC IIa or IIx (Fig. 2a), though atrophy was predominantly observed in the former. One patient showed type 1 fiber uniformity, expressing only MYHC I antibody and no MYHC IIa or IIx (Fig. 2b).

ZASP p.V566M mutation

The V566 M missense mutation in the *ZASP* gene occurred in an affected woman (Patient 7) who first showed signs of slowly progressive weakness with distal dominance at ~40 years old and was 63 years old at the time of biopsy (Table 2). Although her clinical features were similar to those associated with DMRV, she was diagnosed as definite IBM according to the criteria [2]. At the time of analysis, the patient did not have, and reported no history of, heart disease; additionally, the patient was unaware of any consanguineous marriages or family history of heart disease and myopathy. Her CK levels were normal and her EMG showed a myogenic pattern. A muscle CT indicated muscle atrophy in the patient's paraspinal muscle and lower limbs, as well as fatty degeneration. Muscle histopathology showed a slightly varied fiber size with slight to moderate rimmed vacuoles. Mild inflammatory infiltration was observed. Immunohistochemical studies of Z-band associated proteins revealed strong accumulation of desmin, NCAM, and β APP in affected fibers, with mild to moderate immunoreactivity for CDC2, myotilin, α -BC, and ubiquitin at the vacuole margins (Fig. 3). Dystrophin C-terminus did not localize consistently to the accumulated aggregates. The results were compatible with MFM by *ZASP* mutation.

Discussion

Of the 5 genes screened during our study of 21 sIBM patients, 2 (*MYHC2A* and *ZASP*) appeared to play a role in generating the sIBM phenotype. Surprisingly, the *GNE* gene showed no mutation in our patients. Thus, despite being the most common causative gene of hIBM, it does not appear to play a role in the pathogenesis of sIBM.

The missense heterozygous *MYHC2A* mutation (p.V805A) identified in 3 of our sIBM patients has not been reported previously—either in Asia or anywhere else. In fact, to our knowledge, only 4 families worldwide have been reported to possess any form of *MYHC2A* mutation. These include a dominant heterozygous missense mutation (p.E706K) identified in a Swedish family [7] and recessive truncating mutations identified in 1 British family (p.Tyr269-Glu302delfsX and p.Arg783X) and 2 Finnish families (p.Glu659-Gly687delfsX11 and p.Leu802X) [12]. The *MYHC2A* missense mutation was first reported in non-familial cases.

This variant was also present in 1 of the 160 control chromosomes. These 80 controls were selected from the DNA database in the Department of Neurology of Hokkaido University Graduate School of Medicine. The majority of controls (n = 76) were healthy; renal failure, cervical spondylosis, sleep apnea due to a small mandible, and suspect neuropathy were present in 1 patient each. The control subject carrying the p.V805A variant was a 52 year old man who had reported numbness in the legs; due to a lack of EMG data or pathology, he was suspected to have neuropathy. During the clinical follow-up to the study, this patient showed no abnormalities on neurological examination and was found to have normal CK levels despite complaining of back pain and myalgia in the legs.

These findings indicate that the p.V805A variant is either pathogenic or a SNP. The former possibility is supported by several pieces of evidence. First, the p.V805A variant is located in the highly evolutionarily conserved domain of the *MYHC2A* gene and causes a change of amino acid sequence; this sort of mutation is commonly pathogenic. Second, while the occurrence of an asymptomatic missense mutation carrier is rare, the sequence variant L1061V has been reported in both normal control and

myopathy patients, suggesting a strong selective pressure against mutations of the *MYHC2A* gene [22]. In some studies, the individuals carrying missense mutations were even observed to be clinically unaffected due to incomplete penetrance and potential genetic modifiers [23, 24]. Third, all 3 p.V805A variant patients were older than the control patient at onset of sIBM (average age of onset among patients = 62 years, vs. age of control patient = 52 years). Thus, it is possible that the control patient will eventually display signs or symptoms of myopathy, joint contractures, and external ophthalmoplegia. Moreover, the control patient's current complaint of back pain and myalgia has also been reported as a common symptom in *MYHC2A* mutant patients.

Immunohistochemical studies of MYHC isoforms may provide important clues for establishing the pathogenicity of the variant. Myosin is a molecular motor protein that transduces chemical energy of ATP hydrolysis into mechanical force, and myosin heavy chain IIa is expressed in fast, ATPase type 2A muscle fibers [12, 18]. Our immunostaining results revealed atrophy or loss of fibers expressing MYHC IIa in 3 patients, a finding that is supported by the results of Tajsharghi et al. [12]. These observations suggest that the p.V805A mutation alters MYHC IIa protein function. Even if the p.V805A variant was presumed to be a SNP of the *MYHC2A* gene, the allele frequency was significantly higher in sIBM patients than in controls ($p < 0.01$), and the *MYHC2A* p.V805A variant significantly increased the risk of sIBM (RR = 12.2).

Our genetic screening also revealed a novel heterozygous missense mutation (p.V566M) in the *ZASP* gene of a patient with distal dominant weakness and sporadic definite IBM. As reported previously, *ZASP* gene mutations in exon 2 (V55I), exon 4 (S196L, T213I), exon 6 (D117N, K136M), exon 10 (I352M), exon 14 (V588I), and exon 15 (D626N) have been associated with dilated cardiomyopathy, while mutations in

exon 6 (A147T, A165V), exon 9 (R268C), and exon 15 (D626N) cause myopathy with or without cardiomyopathy [16, 25, 26]. To date, p.V566M has not been associated with either cardiomyopathy or myopathy.

ZASP is a sarcomeric protein expressed in human cardiac and skeletal muscle at the Z-band. All ZASP isoforms have an N-terminal PDZ domain from exon 1 to exon 3, a 26-residue ZASP-like motif (ZM motif) from exon 4 to exon 6, and 3 LIM domains transcribed from the remaining exons. Because of splice variants, not all exons are transcribed in cardiac and skeletal muscles, and 3 transcripts appear in skeletal muscle [16]. Exon 15 is present only in the 2 long transcripts; the mutation in exon 15 (D626N) increases the affinity of the LIM domain for protein kinase C [27]. The novel mutation described here is found in a highly evolutionarily conserved domain in exon 15. We only observed this mutation in a single distal myopathy patient and in none of the control chromosomes. Cumulatively, our results indicate that the V566M mutation alters the functionally important ZASP protein, causing abnormalities in the Z-band. Because it appears that the V566M mutation was responsible for the disease observed in this patient, we revised her diagnosis to zaspopathy from definite IBM.

The criteria of Needham and Mastaglia are currently used when diagnosing IBM. According to these guidelines, both the core characteristic features of symptoms and muscle biopsy data including immunohistochemical results are mandatory when making a definite diagnosis [2]. Since the immunohistochemical staining is not normally performed during muscle pathological studies, medical practice is prone to emphasize the core features of adult-onset muscle weakness and the cardinal pathological findings from light microscopy (e.g., rimmed vacuoles, inflammatory infiltration, and intracellular amyloid deposits detected by Congo red or crystal violet stains) [28], with

the immunohistochemical findings viewed solely as reference. In following the criteria of Needham and Mastaglia, we performed immunohistochemical staining and were able to diagnose 14 patients with definite IBM, 3 patients with probable IBM, and 4 patients with possible IBM. Of note, the 3 patients with *MYHC2A* variant and the patient with ZASP mutation were all diagnosed with definite IBM. It may be difficult to accurately distinguish between IBM, familial zaspopathy, and hIBM. Since the criteria of Needham and Mastaglia may not be sufficient to exclude certain overlapping disorders, other diagnostic criteria of IBM are important. The 3 patients with mutations in *MYHC2A* and the zaspopathy patient were all diagnosed as definite IBM according to Griggs criteria, as well [28]. However, according to the European Neuromuscular Center (ENMC) criteria, the 3 patients with mutations in *MYHC2A* were definite IBM, while the zaspopathy patient was possible IBM [29]. The zaspopathy patient did not show a classical IBM phenotype, since the quadriceps femoris and finger flexor muscles are more affected and the hamstring muscles are less affected in the classical IBM phenotype. Taken together, these findings call attention to the possibility of the non-familial cases carrying the mutations. Meanwhile, the immunohistochemical studies and causative gene screening would be further emphasized in the diagnostic procedure of IBM.

Unfortunately, we were only able to recruit 21 patients into our study; this relatively small cohort may have biased our results. We were also unable to procure adequate DNA and muscle specimens for performing genetic and immunohistochemical studies on all patients. To some extent, this limited our ability to apply the criteria of Needham and Mastaglia. As a result of these limitations, we recommend further studies

utilizing larger sample sizes in order to confirm the *MYHC2A* variant and elucidate its role in the pathogenesis of, or the susceptibility to, sIBM.

In conclusion, we have identified a novel *ZASP* mutation and a novel *MYHC2A* variant, thereby expanding our understanding of the molecular background of sIBM. Moreover, investigation of the genotype and phenotype also provided good experience of the application of the IBM criteria of Needham and Mastaglia. Further clinicopathological studies will be needed across a large cohort in order to clarify the importance of genetic susceptibility factors and causative genetic mutations in the pathogenesis of sIBM. In particular, future efforts should investigate additional candidate genes, including *α -B crystallin*, *dystrophin*, and *myotilin*.

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Conflicts of interest: None

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LEGENDS

Figure 1. Sequence chromatograms of the c.2542T>C (p.V805A) mutation in the *MYHC2A* gene (a) and the c.1719G>A (p.V566M) mutation in the *ZASP* gene (b). Evolutionarily conserved domains of the *MYHC2A* p.V805A mutation (c) and the *ZASP* p.V566M mutation (d).

Figure 2. Immunohistochemistry for MYHC isoforms of *MYHC2A* p.V805A patients. The immunohistochemistry shows dark brown fibers expressing slow MYHC I (ATPase Type 1 fibers), dark pink fibers expressing MYHC IIa (ATPase Type 2A fibers), and light pink fibers expressing MYHC IIx (ATPase Type 2B fibers). Immunohistochemistry staining shows atrophy of fibers that express either MYHC IIa or IIx, with predominant atrophy of the former (a). One patient showed Type 1 fiber uniformity, expressing only slow MYHC I and no MYHC IIa or IIx (b). Scale bars = 100 μ m.

Figure 3. Light microscope pictures of quadriceps muscle biopsy of the *ZASP* p.V566M patient. A modified Gomori trichrome stain demonstrating a slightly varied fiber size with rimmed vacuoles (white arrow) (a). Note strong accumulation of desmin (b), NCAM (c), and β APP (d) in affected fibers, and mild to moderate immunoreactivity for CDC2 (e), myotilin (f), α -BC (g), and ubiquitin (h) in the vacuole margins. Immunoreactivity is indicated by black arrows (b, c, d, e, f, g, and h). Scale bars = 20 μ m (a) and 25 μ m (b, c, d, e, f, g, and h).

Table 1. The clinical features and causative genes of familial inclusion body myopathies

Type	OMIM no	Clinical features	Gene & locus
IBM1	# 601419	Distal muscle weakness and cardiomyopathy	<i>Desmin</i> 2q35
IBM2	#600737	Distal muscle weakness in the lower limbs with the quadriceps muscle being spared	<i>GNE</i> 9p13.3
IBM3	#605637	Proximal muscle weakness, joint contractures, and external ophthalmoplegia	<i>MYHC2A</i> 17p13.1
IBMPFD	#167320	Inclusion body myopathy with early-onset Paget disease of the bone, and frontotemporal dementia	<i>VCP</i> 9p13.3
Zaspopathy	#609452	Distal or proximal muscle weakness with or without cardiomyopathy	<i>ZASP</i> 10q23.2

OMIM no , OMIM number; IBM1, inclusion body myopathy 1; IBMPFD, inclusion body myopathy with Paget disease and frontotemporal dementia; GNE, UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase; MYHC2A myosin heavy chain IIa ; VCP, valosin-containing protein; ZASP, Z-band alternatively spliced PDZ-motif containing protein

Table 2. Clinical features of the 21 IBM patients

Pt	Sex/Age at onset/ Age at biopsy (years)	Distribution of affected muscles	Proximal vs. distal	CK	Ald	EMG	Biopsy site
1	M/62/65	Qua, Ilio, Fin	P>D	1103	13	N	Qua
2	F/59//68	Qua, Bi, Fin	P>D	192	1.6	NA	Bi
3	M/52/59	Qua, Bi, Fin	P>D	1096	9.4	M	Qua
4	F/73/74	Qua, Ilio, Bi, Fin	P>D	571	6.3	Mix	Bi
5	M/52/54	Qua, Fin	P=D	336	6.0	NA	Qua
6	F/45/47	Qua, Bi	P	535	8.52	NA	Bi
7	F/40/63	Qua, Ilio, Ham, TA	D>P	114	0.8	M	Qua
8	M/63/68	Qua, Bi	P	543	12.5	N	Bi
9	M/70/75	Qua, Fin	P=D	368	NA	M	Qua
10	F/67/72	Qua, Bi, Fin	P>D	576	1.5	NA	Bi
11	F/39/44	Qua, Del, Bi, Fin	P=D	NA	NA	NA	Qua
12	M/66/68	Qua, Ilio, Bi, Fin	P>D	483	1.2	NA	Bi
13	F/60/67	Qua	P	865	NA	Mix	Qua
14	M/35/37	TA, Fin	D>P	1768	NA	NA	Qua
15	F/47/55	Qua, Bi, Fin	P=D	417	6.2	N	Bi
16	M/69/72	Qua, TA, Del, Bi, Tri	P=D	517	3.6	M	Bi
17	M/58/68	Qua, Ilio, Fin	P=D	429	7.2	NA	Qua
18	M/68/70	Qua, Bi, Fin	P>D	579	NA	M	Qua
19	M/68/73	Qua, TA, Bi, Tri, Fin	P>D	305	3.7	M	Bi
20	F/55/59	Qua, Ilio	P	611	6.9	M	Qua
21	M/70/74	Qua, Fin	P>D	1118	14.4	M	Qua

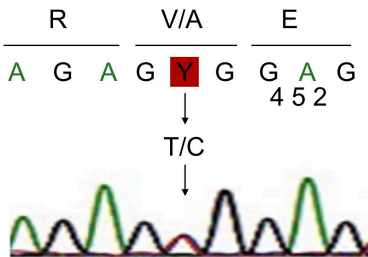
IBM, inclusion body myositis; Pt, patient; Qua, quadriceps femoris; Ilio, iliopsoas; Fin, finger flexor muscles; Bi, biceps brachii; Ham, hamstring muscles; TA, tibialis anterior; Del, deltoid; Tri, triceps brachii; CK, creatine kinase; Ald, aldolase; EMG, electromyogram; M, myogenic pattern; N, neurogenic pattern; Mix, mixed pattern; NA, not available

1 **Table 3.** Pathological findings and genetic analysis of the 21 IBM patients

Pt	Pathological findings							Diagnosis	Genetic analysis
	RV	I Inf	βAPP	CD8 ⁺	MHC-I	COX-	Ubi		
1	+	+	+	NA	NA	NA	NA	Probable	-
2	+	+	+	NA	NA	NA	NA	Probable	-
3	+	+	+	+	+	+	+	Definite	<i>MYHC2A</i> p.V805A
4	+	+	-	+	+	+	+	Probable	-
5	+	+	+	+	+	+	+	Definite	-
6	+	+	+	+	+	+	-	Definite	-
7	+	+	+	+	+	+	+	Definite	<i>ZASP</i> p.V566M
8	+	+	+	NA	NA	NA	NA	Possible	-
9	+	+	+	+	+	+	+	Definite	-
10	+	+	+	+	+	+	+	Definite	<i>MYHC2A</i> p.V805A
11	+	+	+	+	+	+	+	Definite	-
12	+	+	+	+	+	+	+	Definite	<i>MYHC2A</i> p.V805A
13	+	+	+	NA	NA	NA	NA	Possible	-
14	+	+	+	+	+	+	+	Definite	-
15	+	+	+	+	+	+	+	Definite	-
16	+	+	+	NA	NA	NA	NA	Possible	-
17	+	+	+	+	+	+	+	Definite	-
18	+	+	+	+	+	+	+	Definite	-
19	+	+	+	+	+	+	+	Definite	-
20	+	+	+	+	+	-	-	Possible	-
21	+	+	+	+	+	+	+	Definite	-

2 Pt, patients; RV, rimmed vacuoles; I Inf, inflammatory infiltration; CD8⁺, CD8⁺ T cell; βAPP, β-amyloid peptide
3 precursor; MHC-I, upregulation of major histocompatibility complex (MHC)-I expression; COX-, cytochrome c
4 oxidase (COX) negative fibers; Ubi, ubiquitin positive; NA, not available

a **MYHC2Ac.2542T>C(p.V805A)**



c Human (p. V805A)

Pongo abelii

Macaca mulatta

Monodelphis domestica

Oryctolagus cuniculus

Sus scrofa

Mus musculus

Canis familiaris

d Human (p. V566M)

Pongo abelii

Macaca mulatta

Monodelphis domestica

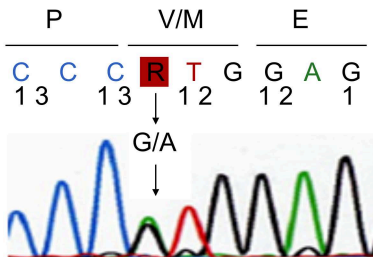
Oryctolagus cuniculus

Sus scrofa

Mus musculus

Canis familiaris

b ZASPC.1719G>A(p.V566M)



RTQARCRGFLARVEYQRMVERRE

RTQARCRGFLAR**V**EYQRMVERRE

RTQARCRGFLAR**V**EYQRMVERRE

RTQARCRGFLAR**V**EYQRMVERRE

RTQARCRGFLARVEYQRMVERRE

RTQARCRGFLAR**V**EYQRMVERRE

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