学位論文内容の要旨
(Summary of dissertation)

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学位論文題名
(Title of dissertation)
Clinical, pathological, and genetic analysis of
sporadic inclusion body myositis in Japanese people
（日本人の封入体筋炎における臨床症状、筋病理所見と遺伝学的背景に関する研究）

【Background and Objectives】 Inclusion body myositis (IBM) is a common adult-onset, sporadic muscle disease with pathological findings of rimmed vacuoles, mononuclear cell invasion of non-necrotic fibers, and amyloid deposits or 15–18 nm filaments on electron microscopy. However, rimmed vacuoles are not specific to IBM and may be observed in inherited vacuolar myopathies such as hereditary inclusion body myopathy (hIBM) and myofibrillar myopathy (MFM). In general, clinical features of IBM may be diverse and show some overlap with other vacuolar myopathies. Molecular genetic studies have identified several genetic loci associated with some inherited vacuolar myopathies: 3 genes for hIBMs (*Desmin*, *GNE*, and *MYHC2A* genes), the *VCP* gene for inclusion body myopathy with early-onset Paget disease of the bone and frontotemporal dementia (IBMPFD), the *ZASP* gene for zaspopathy (one form of MFM) and the *DNAJB6* gene for limb-girdle muscular dystrophy 1D (LGMD1D). However, the extent to which these genes play a role in generating the phenotype of IBM is poorly understood. In order to explore the molecular basis of IBM and to investigate genotype-phenotype correlations, we performed a clinicopathological analysis of 23 IBM patients and screened for mutations in the above 6 candidate genes.

【Methods】 According to the Needham and Mastaglia criteria, 23 suspect IBM patients were recruited from a total of 692 cases with available muscle pathology from December 1993 to December 2011. 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 4.2, 4.6, and 10.4), non-specific esterase, and alkaline phosphatase, as well as β-amyloid peptide precursor protein (βAPP) immunostaining. Cytochrome c oxidase (COX) studies and immunostaining for $CD8^+$ T cells and MHC-I expression could only be performed in 18 patients due to insufficient specimen samples. Double immunostaining for MYHC isoforms was performed in 3 patients with *MYHC* mutation. Immunohistochemical staining of Z-band associated proteins was performed in 2 patients with a *ZASP* mutation or variant. After extracting DNA from blood or frozen muscle, PCR was used to amplify all coding exons of the *Desmin GNE*, *MYHC2A*, *VCP*, *ZASP*, and *DNAJB6* genes, as well as the flanking non-coding regions of the above**.** Direct sequencing of the *DNAJB6* gene was performed in 20 patients due to insufficient DNA, while sequencing of other 5 genes was performed in all patients.

【Results】 According to the Needham and Mastaglia criteria, 15 patients were diagnosed with definite IBM, 3 patients with probable IBM, and 5 patients with possible IBM. No cases showed missense mutations in the *Desmin*, *VCP,* or *DNAJB6* genes. Three patients with IBM carried the missense mutation p.V805A in the *MYHC2A* gene. The p.V805A variant was only present in 1 of 160 control chromosomes. The frequency of this allele was significantly higher in the IBM patients than in the controls (6.5% vs. 0.6%, respectively; $p < 0.05$). Further, the p.V805A variant in the *MYHC2A* gene was associated with a significantly increased risk of IBM (Odds Ratio = 11.1; 95% CI = 1.13-109.3). The immunohistochemical staining for MYHC isoforms in these 3 cases showed atrophy or loss of muscle fibers expressing MYHC IIa or IIx. One patient with IBM harbored the missense mutation p.V566M in the *ZASP* gene. The mutation was not present in 160 control chromosomes. Immunohistochemical studies of Z-band associated proteins revealed strong accumulation of desmin, dystrophin C-terminus, neural cell adhesion molecule (NCAM), and βAPP in affected fibers, with mild to moderate immunoreactivity for cell division cycle 2 (CDC2), myotilin, α-B crystalline (α-BC), and ubiquitin at the vacuole margins. The immunohistochemical studies of Z-band associated proteins revealed typical Z-band abnormalities, indicating that the V566M mutation alters the functionally important ZASP protein. One patient with possible IBM was identified to carry both the *GNE* missense mutations and the *ZASP* variant: novel compound heterozygous missense mutations in the *GNE* gene (p.V421A and p.N635K) and a heterozygous variant in the *ZASP* gene (p.D673N). Both the *GNE* mutations were not observed in 160 control chromosomes while the *ZASP* p.D673N variant was identified in 2 of 160 control chromosomes. Immunohistochemical analysis of Z-band associated proteins showed mild to moderate immunoreactivity to βAPP, NCAM, α-BC, and ubiquitin, and no immunoreactivity to desmin, CDC2, and myotilin, indicating atypical features of *ZASP* associated MFM. All of the mutations were located in highly evolutionarily conserved domains of their respective genes.

【Discussion】 Of the 6 genes screened in the 23 IBM patients, we identified 5 IBM patients carrying missense mutations. The 3 patients with *MYHC2A* p.V805A mutation displayed proximal dominance of limb weakness with involvement of the quadriceps femoris and finger flexors, and showed both clinical and pathological phenotypes resembling that of IBM. The diagnosis remained considerably indistinguishable between IBM and *MYHC2A* associated IBM3. However, the immunostaining results of MYHC isoforms revealed atrophy or loss of fibers expressing MYHC IIa in 3 patients, suggesting the p.V805A mutation alters MYHC IIa protein function and p.V805A mutation is pathogenic in 3 patients. The patient with *ZASP* V566M mutation displayed distal dominance of limb weakness with the tibialis anterior being more affected than the quadriceps femoris, indicating this patient did not show a classical IBM phenotype. The typical findings of the Z-band associated protein immunostaining were compatible with *ZASP* associated MFM. It appears that the V566M mutation was responsible for this patient, and the diagnosis was revised as zaspopathy from IBM. The patient with *GNE* mutations and *ZASP* variant presented with distal weakness sparing the quadriceps muscles, and showed atypical pathological findings of IBM lacking mononuclear cell invasion of non-necrotic fibers and upregulation of MHC class I expression. Moreover, the atypical findings of the Z-band associated protein immunostaining did not compatible with *ZASP* associated MFM. It seems that the *GNE* mutations are pathogenic and the diagnosis was revised as IBM2/DMRV from possible IBM.

【Conclusion】Cumulatively, approximately 20% of the IBM patients in this study harbored missense mutations, and 3 genes (*MYHC2A*, *ZASP*, and *GNE*) appeared to play a role in generating the phenotype of IBM. These findings highlight 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. Moreover, we suggest that typical clinical features and typical pathological findings, including $CD8^+$ T cell invasion of non-necrotic fibers, are both required for accurately diagnosing IBM.