Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 313 (2004) 178–184
BBRC
www.elsevier.com/locate/ybbrc

Identification and functional analysis of a caveolin-3 mutation associated with familial hypertrophic cardiomyopathy

Takeharu Hayashi,[a] Takuro Arimura,[a] Kazuo Ueda,[a] Hiroki Shibata,[a] Shigeru Hohda,[a] Megumi Takahashi,[a] Hisae Hori,[a] Yoshinori Koga,[b] Naoki Oka,[c] Tsutomu Imaizumi,[c] Michio Yasunami,[a,d] and Akinori Kimura[a,d,*]

[a] *Department of Molecular Pathogenesis, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan*
[b] *Department of Cardiology, Kurume Medical Center, Kurume University, Kurume 835, Japan*
[c] *Third Department of Internal Medicine, Kurume University School of Medicine, Kurume 830-0011, Japan*
[d] *Laboratory of Genome Diversity, School of Biomedical Science, Tokyo Medical and Dental University, Tokyo, Japan*

Received 10 November 2003

Abstract

Hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) are caused by mutations in 14 and 15 different disease genes, respectively, in a part of the patients and the disease genes for cardiomyopathy overlap in part with that for limb-girdle muscular dystrophy (LGMD). In this study, we examined an LGMD gene encoding caveolin-3 (CAV3) for mutation in the patients with HCM or DCM. A Thr63Ser mutation was identified in a sibling case of HCM. Because the mutation was found at the residue that is involved in the LGMD-causing mutations, we investigate the functional change due to the Thr63Ser mutation as compared with the LGMD mutations by examining the distribution of GFP-tagged CAV3 proteins. It was observed that the Thr63Ser mutation reduced the cell surface expression of caveolin-3, albeit the change was mild as compared with the LGMD mutations. These observations suggest that HCM is a clinical spectrum of CAV3 mutations.

Keywords: Caveolin-3; Hypertrophic cardiomyopathy; Limb-girdle type muscular dystrophy; mutation

Because idiopathic cardiomyopathy is one of the major causes of severe heart failure and sudden death in young, it is important to elucidate the etiology and pathogenesis. Hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) are two major clinical phenotypes of the idiopathic cardiomyopathy. HCM is characterized by left ventricular (LV) hypertrophy accompanied by myofibrillar disarrays and manifested with diastolic dysfunction of cardiac ventricles, while DCM is characterized by dilated ventricular cavity with systolic dysfunction [1].

It has been known that more than half of HCM patients have family history of the disease consistent with autosomal dominant genetic trait, whereas about 20–30% of DCM patients have the family history mainly consistent with the autosomal dominant inheritance [2]. Recent molecular genetic approaches have revealed that HCM is caused by mutations in the genes for sarcomeric proteins, including *MYH7* (cardiac myosin heavy chain), *TNNT2* (cardiac troponin T), and *TTN* (titin) [2,3]. On the other hand, mutations in the sarcomere genes including *MYH7*, *TNNT2*, and *TTN* also cause DCM [4–6] in addition to the mutations in the genes for sarcolemma or Z-disc related proteins, such as *DMD* (dystrophin), *DES* (desmin), and *SGCD* (δ-sarcoglycan) [7–9]. We have recently reported that the mutations in *MLP* and *TCAP*, both being the Z-disc protein genes, cause DCM [10]. However, mutations in these disease-causing genes could be found in less than half of HCM and in a few percent of DCM, at least in Japanese [11].

It is quite interesting that mutations in *DES*, *SGCD*, *DMD*, and *TCAP* were also reported to cause muscular dystrophy [12–15]. In addition, a *TTN* mutation is recently reported as a cause of tibial muscular dystrophy [16]. These observations demonstrated that the disease

* Corresponding author. Fax: +81-3-5280-8055.
E-mail address: akitis@mri.tmd.ac.jp (A. Kimura).

0006-291X/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2003.11.101

genes for cardiomyopathy overlapped in part with the disease genes for muscular dystrophy. Therefore, we reasoned to search for mutations in caveolin-3 (*CAV3*) gene, a disease-causing gene for autosomal dominant limb-girdle muscular dystrophy (LGMD) type 1C [17], in the Japanese patents with HCM or DCM. *CAV3* mutations are also reported to cause rippling muscle disease and asymptomatic hyperCKemia [18,19].

CAV3 encodes a raft protein, caveolin-3, a component of caveolae [20]. Caveolin-3 is expressed in cardiac, skeletal, and smooth muscles and interacts at the scaffolding domain with nNOS, PKC, and G proteins that are related to the hypertrophic signal [20]. Caveolin-3 also interacts with angiotensin II type 1 receptor that plays an important role in cardiac hypertrophy [21], and the overexpression of *CAV3* inhibits phenylephrine- and endothelin-1-induced hypertrophy of cardiomyocytes [22]. These findings suggested that caveolin-3 might be involved in the pathogenesis of cardiomyopathy, especially for HCM. Indeed, caveolin-3 knockout mice showed hypertrophy of cardiomyocytes at 2 months of age, and manifested with left ventricular hypertrophy, chamber dilation, and decrease in fractional shortening at 4 months of age [23]. These phenotypes are similar to those observed in HCM patients.

In this study, a novel *CAV3* mutation was found in an HCM family, and functional change due to the mutation was investigated along with the LGMD-causing mutations. This is the first report of the *CAV3* mutation in cardiomyopathy not associated with the skeletal muscle phenotypes.

Material and methods

Subjects. One hundred and forty-six and one hundred and thirty genetically unrelated index patients with HCM and DCM, respectively, were the subjects. Apparent family histories of the disease consistent with autosomal dominant inheritance were observed in all HCM cases and in 32 DCM cases. All the index patients were diagnosed to have idiopathic cardiomyopathy as described previously [3,6,11]. These patients were selected for this study since no mutation in the known disease genes was identified in them. The DCM patients had been analyzed for mutations in the α-cardiac actin, desmin, dystrophin, lamin A/C, MLP, titin, and Tcap genes, while the HCM patients were searched for mutations in the β-myosin heavy chain, cardiac troponin T, α-tropomyosin, cardiac myosin binding protein-C, myosin regulatory light chain, myosin essential light chain, cardiac troponin I, cardiac α actin, MLP, titin, and Tcap genes [6,10,11]. Family relatives of the index patient carrying a *CAV3* mutation were also examined for the mutation and clinical status. Control subjects were 260 unrelated healthy Japanese individuals selected at random. Blood samples were obtained from each subject after a given informed consent. The study protocol was approved by the Ethics Reviewing Committee of the Medical Research Institute, Tokyo Medical and Dental University.

Mutational analysis of CAV3. Genomic DNA was extracted from peripheral blood from each subject and subjected to PCR under standard conditions by use of primer pairs specific to each region analyzed. Sequence information for the primers is as follows. 1F, 5′-AGCTCGGATCTCCTCCTGTG-3′; 1R, 5′-AAACCTGACACTCTCCGCCC-3′; 2F, 5′-GTGGCTTCTGTGAGTTGAGG-3′; 2inR, 5′-ATGGCACCACCGCCCAGAT-3′; 2inF, 5′-TCTGTTGTCCACGCTGCTGG-3′; and 2R, 5′-CCTGCCCTGCCACCGCTGTT-3′. The PCR was separately done with combinations of primers, 1F and 1R, 2F and 2inR, and 2inF and 2R. The PCR products from patients were searched for sequencing variations using the PCR-SSCP methods, as described previously [24]. When an abnormal SSCP pattern was observed, the PCR product was cloned into pCRII vector (Invitrogen) and several independent clones were sequenced on both strands to confirm the sequence variation.

Construction of GFP-tagged caveolin-3 gene. A full-length human caveolin-3 cDNA (GenBank Accession No. AF043101) was amplified by reverse transcription (RT)-PCR from cDNA templates originated from heart, as described previously [6]. The primers used in the RT-PCR method were CAV3-F, 5′-ATGGCAGAAGAGCACACAGA-3′ and CAV3-R, 5′-TTAGACCTCCTTCCGCAGCA-3′. Each mutation was introduced into the cDNA fragment by primer-directed mutagenesis method, as described [6], by using combinations of the following primers, respectively: T63S; CAV3-F and 63S-MF; 5′-TACACCAGCTTCACTGTCTCC-3′ with 63S-MR; 5′-AGTGAAGCTGGTGTAGCTCAC-3′ and CAV3-R, T63P; CAV3-F and 63P-MF; 5′-TACACCCCCTTCACTGTCTCC-3′ with 63P-MR; 5′-AGTGAAGGGGGTGTAGCTCAC-3′ and CAV3-R, del63-65; CAV3-F and DEL63-65MR; 5′-GGAGACGGTGTAGCTCACCTT-3′ with DEL63-65MF; 5′-TACACCGTCTCCAAGTACTGG-3′ and CAV3-R. These fragments were cloned into pcDNA3.1/NT-GFP-TOPO (Invitrogen) and several clones were chosen to confirm the DNA sequence. The cloned cDNAs with or without mutations were confirmed for the sequence and used in the transfection experiment.

Cell culture and transfection to detect GFP signals. COS7 or NIH3T3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. Petri-dishes were plated with 2×10^5 cells and incubated for 24 h. The cells were transfected with 4 μg GFP-tagged *CAV3* constructs using the lipofection (LipofectAMINE; Life Technologies) according to manufacturer's instructions. The cells were cultured for additional 48 h, fixed with 4% paraformaldehyde in PBS buffer at room temperature, and mounted on Vectashield (Vector laboratories). GFP signals were examined under a confocal laser-scanning microscope (Olympus).

Western blot analysis. COS7 cells were transfected with GFP-tagged *CAV3* constructs, incubated for 48 h, washed with PBS, and harvested in 10 mM Tris, pH 8.0, 0.15 M NaCl, and 5 mM EDTA (TBS) containing 1% Triton X-100, with protease inhibitor (Sigma), as described by Song et al. [25]. The cells were harvested and centrifuged at 14,000*g* for 10 min at 4 °C to separate Triton-insoluble membrane fraction (pellet) and Triton-soluble cytoplasmic fraction (supernatant). Equal amount of the pellet and supernatant was applied to SDS–PAGE in 12% polyacrylamide gels and transferred to a nitrocellulose membrane (Schleicher & Schuell). The membrane was blocked with 3% non-fat dried milk and incubated for overnight at 4 °C with N-terminal caveolin-3 gout IgG polyclonal antibody (Santa Cruz). After washing with TBS containing 0.05% Tween 20, the membrane was incubated with alkaline phosphatase-conjugated donkey anti-gout IgG antibody (Promega) as a second antibody for 1 h and stained by alkaline phosphatase conjugated kit (Bio-Rad). These assays were repeated at least for 5 times, and each blot was analyzed by NIH image to measure the amount of wild type or mutant caveolin-3 in the membrane and cytoplasmic fractions.

Results

Identification of a CAV3 mutation in patients with HCM

Sequence variations in *CAV3* were searched for in the Japanese patients with HCM and DCM using the

PCR-SSCP method. Three variations not accompanied by amino acid replacement, nucleotide changes of C to T, C to A, and C to A, at nucleotide position of 96, 165, and 201, respectively (nucleotide position from AF043101), and a missense mutation in exon 2 were found in the patients. The former variations were also found in the healthy controls at a similar frequency, showing that they were polymorphisms not associated with the cardiomyopathy. In clear contrast, the missense mutation was detected in a male index patient with HCM (CM39) as an abnormal SSCP pattern (Fig. 1A). This SSCP pattern was not found in more than 500 control chromosomes, and the sequence analysis revealed that it was due to a C to G transition in codon 63, replacing ACC (Thr) with AGC (Ser) (T63S) (Fig. 1B). Because both normal and mutant sequences were obtained from the patient, he was heterozygous for the mutation. Other parts of *CAV3* from the index patient were also sequenced to confirm that he had no other mutations in *CAV3* (data not shown). The mutation was located in the scaffolding domain that was crucial for interaction with cardiac hypertrophy-associated signaling molecules. The mutation was also found in his younger brother (CM40) affected with HCM and was not found in their mother who did not show LV hypertrophy (Fig. 1A). It was, then, suggested that the

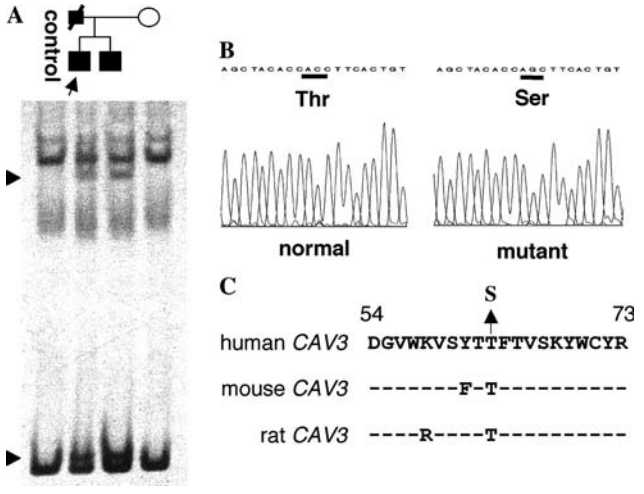

Fig. 1. Mutational analysis of the caveolin-3 gene. (A) PCR-SSCP analysis of exon 2. Lane 1 was a healthy control and lanes 2 and 3 were sib-cases of HCM. A pedigree of the patients is shown, and the abnormal band pattern represented by filled triangles was found in the proband patient (CM 39, lane 2) and his affected brother (CM40, lane 3). Since the normal bands were found in the patients, they were heterozygous for the mutation. Their father had died suddenly at the age of 41. Their mother had no cardiac disease and no abnormal SSCP pattern (lane 4). (B) Nucleotide sequence of normal and mutant alleles obtained from CM39, showing that he was heterozygous for the mutation. Codon 63 of normal allele was ACC (Thr), while that of mutant allele was AGC (Ser). (C) Alignment of caveolin-3 amino acid sequences at a scaffolding domain (codon 54–73) from human and other species. The mutation found in the HCM family was at codon 63 of which threonine residue (Thr) was evolutionary conserved.

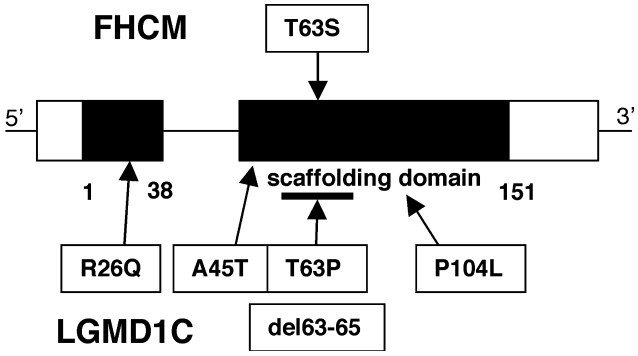

Fig. 2. Schematic representation of exon/intron organization of *CAV3* and variations found in HCM and LGMD1C. In this study, T63S mutation was found in the HCM patients. This mutation was located in the scaffolding domain (line below the exon 2) which was a functionally important domain. Mutations reported in LGMD1C including T63P and del63–65 mutations [17,26] were also shown.

mutation was inherited from their father, but this could not be confirmed since the father who was also affected with HCM had died suddenly at the age of 41 and he had no sibling to be examined for the mutation. The threonine at codon 63 was evolutionary conserved in the scaffolding domain of caveolin-3 (Fig. 1C). Quite interestingly, two mutations involving the codon 63, The63-Pro (T63P) and a deletion of 3 amino acids at positions from 63 to 65 (del63–65) were reported in *LGMD1C* (Fig. 2).

Clinical findings of the index patient, CM39, were mild. At the age of 16, he showed marginal concentric LV hypertrophy (LV wall thickness was 11 mm with septal hypertrophy of apical side), and his LV end-diastolic pressure was high (17 mm Hg) in catheterized pressure study. His electrocardiogram showed high voltage (R wave in V5 lead was 30–34 mm) with apparent ST-T changes (inverted T in V4–V6 leads and mild ST depression and flattened T in limb leads). Following a follow-up of 9 years, although LV wall thickness was not changed remarkably, both LV dilated and systolic dimension were increased. Similar phenotypes were found in CM40. Both of them as well as their father had no symptoms of skeletal muscle disorder and no elevation of serum CK, suggesting that they were not affected with either LGMD, rippling muscle disease, or hyperCKemia.

Functional change of CAV3 due to the mutation

To study the functional alteration of *CAV3* due to the T63S mutation, we constructed a GFP fusion gene for testing the distribution of transfected caveolin-3 molecule. Two LGMD mutations, T63P and del63–65, were also investigated for comparison of functional alterations due to the LGMD-causing CAV3 mutations.

The GFP-CAV3 constructs with or without mutations were transfected into cultured cells and the transiently expressed GFP signals were investigated by

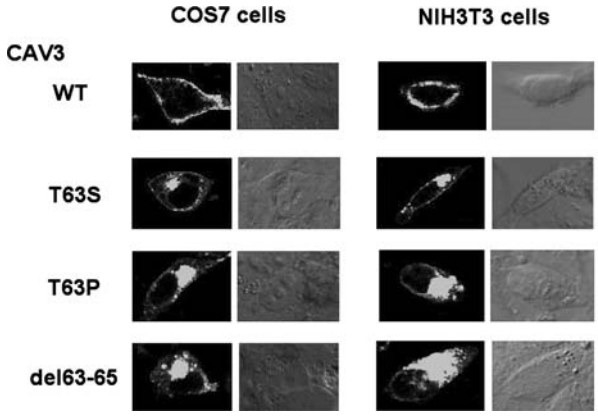

Fig. 3. Fluorescence image of transiently expressed wild type or mutant *CAV3* fused to GFP. Representative results are shown as pair-panels of GFP signal-image and phase-contrast image for each *CAV3* construct. Distribution of GFP-tagged caveolin-3 in the transfected COS7 and NIH3T3 cells was examined under the confocal microscopy. Wild type *CAV3* was expressed almost exclusively at the plasma surface membrane. In contrast, the expression of mutant *CAV3* constructs was decreased at the cell surface and retention in the cytoplasm was observed.

confocal microscopic analysis. In COS7 cells (Fig. 3, left panels), GFP signals fused to the wild type caveolin-3 were expressed almost exclusively at the cell surface. In contrast, signals from both of the LGMD-causing mutants (T63P and del63–65) were observed in the cytoplasm, and the surface expression was remarkably decreased in each case, as has been reported for del63–65 and another LGMD-causing mutation Pro104Leu [20]. On the other hand, the HCM-associated mutant (T63S) showed decreased cell surface expression of GFP signals and retention in the cytoplasm, but the change was to a lesser extent than that found for the LGMD mutants. Similar results were obtained in NIH3T3 cells (Fig. 3, right panels). These findings indicated that the differences in the caveolin-3 distribution were depending on the mutations themselves and not on the cell types.

Because the confocal microscopic analysis was a qualitative assay of the functional alteration of *CAV3* mutations, the change was assessed semi-quantitatively by the Western blot analysis. GFP-tagged *CAV3* constructs were transiently expressed in the COS7 cells. The transfected cells were treated with Triton X-100, and the insoluble (raft) and soluble (cytoplasmic) fractions were separated by the centrifugation as pellet (P) and supernatant (S), respectively. The raft and cytoplasmic fractions were size-fractionated in a SDS–polyacrylamide gel by electrophoresis and analyzed by Western blot assay using anti-caveolin antibody (Fig. 4). The wild type caveolin-3 was almost exclusively distributed in the raft fraction, while all mutant caveolin-3 (T63S, T63P, and del63–65) showed the decreased distribution in the raft fraction and retained in the cytoplasmic fraction. The average ratio of caveolin-3 in the raft and cytoplasmic fractions for each construct is listed in Table 1. The distribution to raft was severely affected by the LGMD-causing mutations, T63P and del63–65, showing $57.6 \pm 2.6\%$ and $47.0 \pm 4.4\%$, respectively, as compared with $97.5 \pm 1.2\%$ of the wild type, whereas it was relatively mild ($75.6 \pm 4.1\%$) for the HCM-associated mutation.

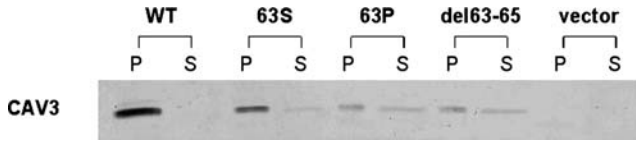

Fig. 4. Western blot analysis of GFP-tagged caveolin-3 proteins expressed in the transfected COS7 cells. Wild type and mutant GFP-tagged *CAV3* constructs were transfected into the COS7 cells. By using Western blot method, the distribution of caveolin-3 in the raft and cytoplasmic fractions was examined. Pellet (P) corresponds to the raft (Triton-insoluble) fraction, while supernatant (S) is derived from the cytoplasmic (Triton-soluble) fraction. Wild type (WT) caveolin-3 was exclusively found in the raft fraction, whereas the mutant (T63S, T63P, and del63–65) caveolin-3 showed decreased expression in the raft fraction and retention in the cytoplasmic fraction. Transfectants of GFP vector alone served as control showing no endogenous expression of *CAV3* in COS7 cells.

Table 1
The average ratio of GFP fused caveolin-3 proteins distributed in the membrane and cytoplasmic fractions

CAV3	Pellet #1 (%)	Supernatant #2 (%)
WT	97.5 ± 1.2	2.0 ± 1.2
T63S	75.6 ± 4.1	24.5 ± 4.3
T63P	57.6 ± 2.6	42.4 ± 2.6
del63–65	47.0 ± 4.4	52.9 ± 4.4

#1, Raft fraction; #2, cytoplasmic fraction.

Discussion

In this study, we identified for the first time a *CAV3* mutation associated with HCM in a sibling case. The mutation, T63S, was found at the evolutionary conserved residue and not found in the healthy controls. Quite interestingly, Thr63 was involved in the other mutations causing LGMD, although the HCM patients with the *CAV3* mutation did not suffer from muscle disorder and showed normal level of serum CK during the follow-up period. It may be worth noting that the clinical phenotypes of the HCM patients were not typical. Nevertheless, these patients showed high voltage in ECG and suggestive diastolic dysfunction with elevated LV end-diastolic pressure. These observations imply that the *CAV3* mutation caused mild HCM in these patients. This is consistent in part with that *CAV3* knockout mice showed concentric left ventricular hypertrophy [23]. Although the cardiac phenotypes of *LGMD1C* patients have so far not been described in

detail, diastolic dysfunction and mild hypertrophy might be seen in the patients, and hence should be evaluated. This may be important because the obligatory carrier of the T63S mutation, father of the HCM sib-cases, had died suddenly at the age of 41.

It was interesting to note that the HCM-related mutation occurred at the same codon as the LGMD mutations. Because the clinical phenotypes of HCM and LGMD are quite different, at least for that skeletal muscle is not obviously affected in HCM, we hypothesized that the functional alterations due to the HCM-associated mutation may be qualitatively or quantitatively different from the LGMD mutations. To test the hypothesis, we investigated the localization or distribution of mutant caveolin-3 by using GFP-tagged CAV3 constructs. The confocal microscopic analysis and Western blot analysis demonstrated that the wild type caveolin-3 was exclusively expressed on the surface plasma membrane. In contrast, both the HCM- and LGMD-related mutations reduced the surface expression, albeit the relatively mild change in the case of the HCM-associated mutant.

The cell surface expression of caveolin-3 was decreased in the muscle from patients with the LGMD mutations, T63P and del63–65 [17,26]. In addition, the LGMD mutation, del63–65, caused decreased surface expression, formed aggregates, and intracellular retention of caveolin-3 in a perinuclear compartment that was identified as the Golgi complex [20]. The expression of LGMD mutants, del63–65 and P104L, reduced the cell surface expression of wild type caveolin-3 by ~80–85%, demonstrating a dominant negative effect of the mutation [20]. In another study, the LGMD-causing mutant caveolin-3 underwent ubiquitination and proteasomal degradation, so that the cell surface expression of total caveolin-3 was further reduced [27]. The loss of caveolin-3 is shown to cause hyperactivation of p42/44 MAPK cascade that plays an important role in the cardiac hypertrophy [23,28]. Moreover, T-tubule systems of caveolin-3 knockout mice were dilated, swollen, and irregularly shaped, suggesting that the dysregulation of muscle calcium homeostasis may be associated with the loss of caveolin-3 [29]. Since we could not obtain biopsy samples of cardiac or skeletal muscle from the HCM patients, the functional and/or pathophysiological changes of caveolin-3 due to the T63S mutation in vivo remain to be clarified. A transgenic mouse introduced with the *CAV3* mutation may resolve the issues.

As for the severity of functional changes due to the mutations, the HCM-associated mutation showed mild dysfunction, while the LGMD-causing mutations exerted severe dysfunction. The difference in the extent of dysfunction might explain the difference in the phenotypes due to the *CAV3* mutations, cardiomyopathy or muscular dystrophy. Muscular dystrophy and cardiomyopathy are often caused by mutations in the same gene, as exemplified by the dystrophin gene mutations. The dystrophin mutations in the N-terminal domains cause DCM, whereas the mutations in the C-terminal domains cause muscular dystrophy [7,30]. Another example is the mutations in titin. We have recently reported that the titin mutations in the cardiac-specific domain (N2-B) and Z-disc domain cause HCM and DCM [3,6], while the titin mutations in the striated muscle-specific domain (N2-A) cause tibial muscular dystrophy [16]. Therefore, cardiomyopathy-related and muscular dystrophy-related mutations in both dystrophin and titin genes have been mapped on the different functional domains. These observations suggested that the difference in the clinical phenotypes, cardiomyopathy and muscular dystrophy, might be explained by that different functional domains were affected.

In this study, however, we found a *CAV3* mutation in HCM at the same domain, exactly at the same codon, as the LGMD-causing mutations. Then, the clinical difference could not be attributed to the difference in the affected functional domain. Our study clearly demonstrated that the HCM-related mutation and muscular dystrophy-related mutations caused dysfunction to different extent. The reason why the relatively mild dysfunction of caveolin-3 affects the cardiac muscle but not the skeletal muscle may be that the cardiac muscle should follow continuous beating composed of contraction and relaxation, while the contraction of skeletal muscle is on demand. Therefore, even a minor functional disturbance in the striated muscles might affect only the cardiac muscle. This is analogous to that homozygous or combined heterozygous mutations leading to loss of Tcap protein cause LGMD [15], whereas a heterozygous missense mutation of *TCAP* causes cardiomyopathy [10]. One may argue that, if a "light" mutation causes solely cardiomyopathy, the "heavy" mutations (for example, LGMD mutations) would affect more severely the cardiac muscle. Indeed, cardiac involvement in the muscular dystrophy is usually manifested as dilated cardiomyopathy, a phenotype sometimes found in late clinical course (burnout phase) of HCM.

In summary, we identified an HCM-associated *CAV3* mutation and evaluated the functional change in comparison with the LGMD-related *CAV3* mutations. The HCM-associated mutation showed qualitatively similar but quantitatively different dysfunction from the LGMD mutations.

Acknowledgments

We thank Drs. H. Toshima, T. Sakamoto, K. Kawai, K. Kawamura, R. Kusukawa, M. Nagano, Y. Nimura, R. Okada, T. Sugimoto, H. Tanaka, H. Yasuda, F. Numano, K. Fukuda, S. Ogawa, A. Matsumori, S. Sasayama, R. Nagai, and Y. Yazaki for their contributions in the clinical evaluation and blood sampling of the index patients with HCM and DCM. This work was supported in part by Grant-in-Aids for

Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan, research grants from the Ministry of Health, Labor and Welfare, Japan, and Mitsui Life Social Welfare Foundation.

References

[1] P. Richardson, W. McKenna, M. Bristow, B. Maisch, B. Mautner, J. O'Connell, E. Olsen, G. Thiene, J. Goodwin, I. Gyarfas, I. Martin, P. Nordet, Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of cardiomyopathies, Circulation 93 (1996) 841–842.

[2] J.G. Seidman, C. Seidman, The genetic basis for cardiomyopathy: from mutation identification to mechanistic paradigms, Cell 104 (2001) 557–567.

[3] M. Satoh, M. Takahashi, T. Sakamoto, M. Hiroe, F. Marumo, A. Kimura, Structural analysis of the titin gene in hypertrophic cardiomyopathy: identification of a novel disease gene, Biochem. Biophys. Res. Commun. 262 (1999) 411–417.

[4] M. Kamisago, S.D. Sharma, S.R. DePalma, S. Solomon, P. Sharma, B. McDonough, L. Smoot, M.P. Mullen, P.K. Woolf, E.D. Wigle, J.G. Seidman, C.E. Seidman, Mutations in sarcomere protein genes as a cause of dilated cardiomyopathy, New Engl. J. Med. 343 (2000) 1688–1696.

[5] B. Gerull, M. Gramlich, J. Atherton, M. McNabb, K. Trombitas, S. Sasse-Klaassen, J.G. Seidman, C. Seidman, H. Granzier, S. Labeit, M. Frenneaux, L. Thierfelder, Mutations of TTN, encoding the giant muscle filament titin, cause familial dilated cardiomyopathy, Nat. Genet. 30 (2002) 201–204.

[6] M. Itoh-Satoh, T. Hayashi, H. Nishi, Y. Koga, T. Arimura, T. Koyanagi, M. Takahashi, S. Hohda, K. Ueda, T. Nouchi, M. Hiroe, F. Marumo, T. Imaizumi, M. Yasunami, A. Kimura, Titin mutations as the molecular basis for dilated cardiomyopathy, Biochem. Biophys. Res. Commun. 291 (2002) 385–393.

[7] F. Muntoni, M. Cau, A. Ganau, R. Congiu, G. Arvedi, A. Mateddu, M.G. Marrosu, C. Cianchetti, G. Realdi, A. Cao, M.A. Melis, Deletion of the dystrophin muscle-promoter region associated with X-linked dilated cardiomyopathy, New Engl. J. Med. 329 (1993) 921–925.

[8] D. Li, T. Tapscoft, O. Gonzalez, P.E. Burch, M.A. Quinones, W.A. Zoghbi, R. Hill, L.L. Bachinski, D.L. Mann, R. Roberts, Desmin mutation responsible for idiopathic dilated cardiomyopathy, Circulation 100 (1999) 461–464.

[9] S. Tsubata, K.R. Bowles, M. Vatta, C. Zintz, J. Titus, L. Muhonen, N.E. Bowles, J.A. Towbin, Mutations in the human delta-sarcoglycan gene in familial and sporadic dilated cardiomyopathy, J. Clin. Invest 106 (2000) 655–662.

[10] R. Knoll, M. Hoshijima, H.M. Hoffman, V. Person, I. Lorenzen-Schmidt, M.L. Bang, T. Hayashi, N. Shiga, H. Yasukawa, W. Schaper, W. McKenna, M. Yokoyama, N.J. Schork, J.H. Omens, A.D. McCulloch, A. Kimura, C.C. Gregorio, W. Poller, J. Schaper, H.P. Schultheiss, K.R. Chien, A MLP-telethonin Z disc complex is a key component of the cardiac mechanical stretch sensor machinery, and is defective in a subset of human dilated cardiomyopathy, Cell 111 (2002) 945–955.

[11] A. Kimura, M. Ito-Satoh, T. Hayashi, M. Takahashi, T. Arimura, Molecular etiology of idiopathic cardiomyopathy in Asian populations, J. Cardiol. 37 (Suppl. 1) (2001) 139–146.

[12] D.E. Bulman, S.B. Gangopadhyay, K.G. Bebchuck, R.G. Worton, P.N. Ray, Point mutation in the human dystrophin gene: identification through Western blot analysis, Genomics 10 (1991) 457–460.

[13] V. Nigro, E. de Sa Moreira, G. Piluso, M. Vainzof, A. Belsito, L. Politano, A.A. Puca, M.R. Passos-Bueno, M. Zatz, Autosomal recessive limb-girdle muscular dystrophy, LGMD2F, is caused by a mutation in the delta-sarcoglycan gene, Nat. Genet. 14 (1996) 195–198.

[14] L.G. Goldfarb, K.Y. Park, L. Cervenakova, S. Gorokhova, H.S. Lee, O. Vasconcelos, J.W. Nagle, C. Semino-Mora, K. Sivakumar, M.C. Dalakas, Missense mutations in desmin associated with familial cardiac and skeletal myopathy, Nat. Genet. 19 (1998) 402–403.

[15] E.S. Moreira, T.J. Wiltshire, G. Faulkner, A. Nilforoushan, M. Vainzof, O.T. Suzuki, G. Valle, R. Reeves, M. Zatz, M.R. Passos-Bueno, D.E. Jenne, Limb-girdle muscular dystrophy type 2G is caused by mutations in the gene encoding the sarcomeric protein telethonin, Nat. Genet. 24 (2000) 163–166.

[16] P. Hackman, A. Vihola, H. Haravuori, S. Marchand, J. Sarparanta, J. De Seze, S. Labeit, C. Witt, L. Peltonen, I. Richard, B. Udd, Tibial muscular dystrophy is a titinopathy caused by mutations in TTN, the gene encoding the giant skeletal-muscle protein titin, Am. J. Hum. Genet. 71 (2002) 492–500.

[17] C. Minetti, F. Sotgia, C. Bruno, P. Scartezzini, P. Broda, M. Bado, E. Masetti, M. Mazzocco, A. Egeo, M.A. Donati, D. Volonte, F. Galbiati, G. Cordone, F.D. Bricarelli, M.P. Lisanti, F. Zara, Mutations in the caveolin-3 gene cause autosomal dominant limb-girdle muscular dystrophy, Nat. Genet. 18 (1998) 365–369.

[18] I. Carbone, C. Bruno, F. Sotgia, M. Bado, P. Broda, E. Masetti, A. Panella, F. Zara, F.D. Bricarelli, G. Cordone, M.P. Lisanti, C. Minetti, Mutation in the CAV3 gene causes partial caveolin-3 deficiency and hyperCKemia, Neurology 54 (2000) 1373–1376.

[19] R.C. Betz, B.G. Schoser, D. Kasper, K. Ricker, A. Ramirez, V. Stein, T. Torbergsen, Y.A. Lee, M.M. Nothen, T.F. Wienker, J.P. Malin, P. Propping, A. Reis, W. Mortier, T.J. Jentsch, M. Vorgerd, C. Kubisch, Mutations in CAV3 cause mechanical hyperirritability of skeletal muscle in rippling muscle disease, Nat. Genet. 28 (2001) 218–219.

[20] F. Galbiati, D. Volonte, C. Minetti, J.B. Chu, M.P. Lisanti, Phenotypic behavior of caveolin-3 mutations that cause autosomal dominant limb girdle muscular dystrophy (LGMD-1C). Retention of LGMD-1C caveolin-3 mutants within the Golgi complex, J. Biol. Chem. 274 (1999) 25632–25641.

[21] B.D. Wyse, I.A. Prior, H. Qian, I.C. Morrow, S. Nixon, C. Muncke, T.V. Kurzchalia, W.G. Thomas, R.G. Parton, J.F. Hancock, Caveolin interacts with the angiotensin II type 1 receptor during exocytic transport but not at the plasma membrane, J. Biol. Chem. 278 (2003) 23738–23746.

[22] A. Koga, N. Oka, T. Kikuchi, H. Miyazaki, S. Kao, T. Imaizumi, Adenovirus-mediated overexpression of caveolin-3 inhibits rat cardiomyocyte hypertrophy, Hypertension 42 (2003) 213–219.

[23] S.E. Woodman, D.S. Park, A.W. Cohen, M.W. Cheung, M. Chandra, J. Shirani, B. Tang, L.A. Jelicks, R.N. Kitsis, G.J. Christ, S.M. Factor, H.B. Tanowitz, M.P. Lisanti, Caveolin-3 knock-out mice develop a progressive cardiomyopathy and show hyperactivation of the p42/44 MAPK cascade, J. Biol. Chem. 277 (2002) 38988–38997.

[24] H. Nishi, A. Kimura, H. Harada, H. Toshima, T. Sasazuki, Novel missense mutation in cardiac beta myosin heavy chain gene found in a Japanese patient with hypertrophic cardiomyopathy, Biochem. Biophys. Res. Commun. 188 (1992) 379–387.

[25] K.S. Song, Z. Tang, S. Li, M.P. Lisanti, Mutational analysis of the properties of caveolin-1. A novel role for the C-terminal domain in mediating homo-typic caveolin–caveolin interactions, J. Biol. Chem. 272 (1997) 4398–4403.

[26] C. Matsuda, Y.K. Hayashi, M. Ogawa, M. Aoki, K. Murayama, I. Nishino, I. Nonaka, K. Arahata, R.H. Brown Jr., The sarcolemmal proteins dysferlin and caveolin-3 interact in skeletal muscle, Hum. Mol. Genet. 10 (2001) 1761–1766.

[27] F. Galbiati, D. Volonte, C. Minetti, D.B. Bregman, M.P. Lisanti, Limb-girdle muscular dystrophy (LGMD-1C) mutants of caveolin-3 undergo ubiquitination and proteasomal degradation. Treatment with proteasomal inhibitors blocks the dominant negative effect of LGMD-1C mutation and rescues wild-type caveolin-3, J. Biol. Chem. 275 (2000) 37702–37711.

[28] J. Gillespie-Brown, S.J. Fuller, M.A. Bogoyevitch, S. Cowley, P.H. Sugden, The mitogen-activated protein kinase kinase MEK1 stimulates a pattern of gene expression typical of the hypertrophic phenotype in rat ventricular cardiomyocytes, J. Biol. Chem. 270 (1995) 28092–28096.

[29] F. Galbiati, B. Razani, M.P. Lisanti, Caveolae and caveolin-3 in muscular dystrophy, Trends Mol. Med. 7 (2001) 435–441.

[30] U. Lenk, R. Hanke, U. Kraft, K. Grade, I. Grunewald, A. Speer, Non-isotopic analysis of single strand conformation polymorphism (SSCP) in the exon 13 region of the human dystrophin gene, J. Med. Genet. 30 (1993) 951–954.