Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 357 (2007) 162–167
BBRC
www.elsevier.com/locate/ybbrc

Structural analysis of four and half LIM protein-2 in dilated cardiomyopathy

Takuro Arimura [a], Takeharu Hayashi [a], Yuji Matsumoto [a], Hiroki Shibata [a], Shitoshi Hiroi [a], Takeyuki Nakamura [a], Natsuko Inagaki [a], Kunihiko Hinohara [a], Megumi Takahashi [a], Satoh-Itoh Manatsu [a], Taishi Sasaoka [a,b], Toru Izumi [b], Gisèle Bonne [c,d,e], Ketty Schwartz [c,d], Akinori Kimura [a,*]

[a] *Department of Molecular Pathogenesis, Medical Research Institute, Tokyo Medical and Dental University, Tokyo 101-0062, Japan*
[b] *Department of Internal Medicine and Cardiology, Kitazato University School of Medicine, Kanagawa 225-8555, Japan*
[c] *INSERM U582, Institut de Myologie, Paris F-75013, France*
[d] *Université Pierre et Marie Curie—Paris 6, Faculté de médicine, Paris F-75013, France*
[e] *AP-HP, Groupe Hospitalier Pitié-Salpêtriére, U.F. Myogénétique et Cardiogénétique, Service de Biochimie B, Paris F-75013, France*

Received 6 March 2007
Available online 30 March 2007

Abstract

Dilated cardiomyopathy (DCM) is a cardiac disease characterized by dilated ventricle and systolic dysfunction. Most of the DCM patients are sporadic cases, but a certain population of DCM patients can be familial cases caused by mutations in genes for sarcomere/Z-disc components including titin/connectin. However, disease-causing mutations could be identified only in a part of the familial DCM patients, suggesting that there should be other disease causing genes for DCM. To explore a novel disease gene for DCM, we searched for mutations in *FHL2,* encoding for four and half LIM protein 2 (FHL2) in DCM patients, because FHL2 is known to associate with titin/connectin. A missense mutation, Gly48Ser, was identified in a patient with familial DCM. Functional analysis demonstrated that the *FHL2* mutation affected the binding to titin/connectin. Because FHL2 protein is known to tether metabolic enzymes to titin/connectin, these observations suggest that the Gly48Ser mutation may be involved in the pathogenesis of DCM via impaired recruitment of metabolic enzymes to the sarcomere.

Keywords: Dilated cardiomyopathy; Four and half LIM protein 2; Titin/connectin; Mutation

Dilated cardiomyopathy (DCM) is characterized by ventricular dilation and impaired contractile function [1]. Although the etiology of cardiomyopathy has not been completely elucidated, 20–35% of DCM patients have family history of the disease that is consistent mainly with autosomal dominant inheritance, suggesting that genetic abnormalities cause DCM at least in a part of the patients [2,3], and recent genetic analyses have revealed that DCM can be caused by mutations in the genes for cardiac sarcomere/Z-disc components including titin/connectin [4].

Titin/connectin is expressed in the striated muscles, which is arranged in an anti-parallel manner to span the entire sarcomere, with their amino- and carboxyl-terminal ends in the Z- and M-lines, respectively [5,6]. Titin/connectin appears to be a key component in the assembly, force transmission, and maintenance of resting tension in the sarcomere, and tethers many proteins including half LIM protein 2 (FHL2) in the sarcomere [6,7]. It has been reported that abnormalities in the sarcomeric proteins interacting with titin/connectin cause hereditary DCM [8–12]. In addition, we previously reported DCM-associated mutations in the titin/connectin gene (*TTN*) [13,14], and these DCM-associated mutations decreased the binding to T-cap/telethonin, α-actinin [13]

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

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.03.128

and αB-crystallin [12]. These results imply that impairment of the interaction between titin/connectin and sarcomeric proteins may be a molecular etiology of DCM.

FHL2 is a member of the four and a half LIM domain protein subfamily which possesses an N-terminal half LIM domain followed by four complete LIM domains and preferentially expressed in the heart [15,16]. FHL2 has multiple functions in cell adhesion, focal contact, cell mobility, cytoarchitecture, signal transduction, gene regulation, and apoptosis [17,18], and abrogation of FHL2 exaggerated hypertrophic response to β-adrenergic stimulation [19]. In addition, FHL2 interacts with metabolic enzymes, creatine kinase, phosphofructokinase, and adenylate kinase, suggesting that FHL2 may play an important role in the recruitment of metabolic enzymes to sites of energy consumption in the cardiac sarcomere [20]. We recently reported that a DCM-associated *TTN* mutation, Gln4053ter, decreased the binding to FHL2 [14]. These observations suggest that FHL2 may be involved in the cardiac responses to environmental stress, and the stability of molecular complex of FHL2 and titin/connectin might be important for maintenance of cardiac function.

We report here an *FHL2* mutation identified in a patient with hereditary DCM and its functional alterations, further supporting that impaired interaction of titin/connectin with sarcomere components may be involved in the pathogenesis of DCM.

Materials and methods

Study population. We studied 92 genetically unrelated Japanese patients with DCM. Among them, apparent family histories were observed in 44 cases. All the patients manifested with typical DCM phenotype as described previously [11,21]. Control subjects were 288 unrelated healthy Japanese individuals selected at random. After acquiring informed consent, blood samples were obtained from each subject. The protocol for research was approved by the Ethics Reviewing Committee of Medical Research Institute, Tokyo Medical and Dental University.

Mutational analysis of FHL2 in DCM. Genomic DNA extracted from peripheral blood leukocytes of each panel was subjected to PCR by use of primer pairs specific to the analyzed regions covering whole open reading frame of *FHL2*. PCR conditions and primer information are available upon request. PCR products from each patient were searched for sequence variations by using PCR-SSCP methods, as described previously [22]. When abnormal SSCP patterns were observed, the PCR product was purified by using the Gel Band Purification kit (Amersham-Pharmacia) and directly sequenced on both strands to determine the nucleotide change(s).

Alignment of FHL subfamilies from various species. Protein sequence of human FHL2 predicted from nucleotide sequence (GenBank Accession No. NM_201555) was aligned with those of bovine (BC112720), rat (NM_031677), mouse (AF153340), and zebrafish (BC078393), along with FHL1 sequences from human (NM_001449), orangutan (CR858833), macaque (AB168906), bovine (BC102083), pig (AJ275968), *Xenopus* (NM_001006702), and zebrafish (NM_001007287). FHL3 sequences from human (NM_004468), bovine (NM_001034223), and *Xenopus* (NM_001008164) were also aligned with FHL2 sequences. The predicted translation from mRNA and their alignments were performed using DNASIS Software (Hitachi).

Mammalian two-hybrid assay to measure binding of titin/connectin with FHL2. We obtained cDNA fragments of human *TTN* and *FHL2* by RT-PCR from human adult heart cDNA. Wild-type (WT) cDNA fragment encoding titin/connectin N2B region was previously described [12]. WT cDNA fragments of titin/connectin is2 region (from bp76303 to bp77751 of X90568 corresponding aa25391–aa25871) and *FHL2* (from bp122 to bp961 of U29332 corresponding aa1–aa280) were amplified using primers: TTNis2F (5′-GTCGACTTCTGAGGTACAAGAAACAGGAATTC-3′, underlined sequences are added for cloning) and TTNis2R (5′-GCGGCCGCCAGTGTGAAAGGCTGCTGAC-3′); and FHL2F (5′-GTCGACTTATGACTGAGCGCTTTGACTG-3′) and FHL2R (5′-GCGGCCGCTCAGATGTCTTTCCCACAGTC-3′), respectively. Mutant cDNA fragment of *FHL2* carrying a G to A substitution introduced by primer-directed mutagenesis method was obtained by combination of two PCR products using primers: FHL2F and FHL2-48MutR (TGTGGGAAGCCCAT CaGCTG, lowercase letter is the mutation to be introduced); and FHL2–48MutR (CCTTGCAGTCACAGCtGATG) and FHL2R. All cDNA fragments were cloned into pCRII (Invitrogen) and excised by digestion by *Sal*I and *Not*I. The excised cDNA fragments were cloned into the p-ACT vector containing pVP16 as a prey (for *TTN* cDNAs) and the p-BIND vector containing pGAL4 as a bait (for *FHL2* cDNAs) (CheckMate Mammalian two-hybrid system, Promega). These constructs were sequenced to ensure that no errors were introduced. The M2H assays were performed as described previously [12].

Immunoprecipitation. COS-7 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C with 5% CO_2. Cells were seeded at 1.0×10^6 in 100 mm dishes 24 h before transfection and 5 μg each of p-ACT-based plasmids and p-BIND-based plasmids were co-transfected with 20 μl of COSFectin Lipid Reagent (Bio-Rad). Transfected cells were harvested after 72 h, permeabilized and subjected to brief sonication in 250 μl of TNE buffer (1% NP-40, 1 mM EDTA, 150 mM NaCl, and 10 mM Tris–HCl, pH 7.8) with protease inhibitor cocktail (Sigma–Aldrich). Cell debris was removed by centrifugation and supernatant was pre-cleaned by incubation at 4 °C with protein G–Sepharose beads (Amersham-Pharmacia), followed by centrifugation. After measuring protein concentration by using BCA protein assay regent (Pierce Biotechnology), aliquots were removed for use of direct immunoblotting and remaining supernatants containing equal amount of proteins were incubated overnight at 4 °C with 6 μg of mouse anti-VP16 monoclonal antibody (Ab) (Santa Cruz Biotechnology). Protein G–Sepharose beads were then added and incubated for 1 h. The beads were washed with TNE buffer and bound proteins were eluted in 2× Laemmli buffer. Eluted samples were boiled for 5 min, separated on SDS–PAGE gels and transferred to a nitrocellulose membrane. After a pre-incubation with 3% skim milk in PBS, the membrane was incubated with primary rabbit anti-GAL4 polyclonal Ab or anti-VP16 monoclonal Ab, and with secondary rabbit anti-mouse (for monoclonal Ab) or goat anti-rabbit (for polyclonal Ab) IgG HRP-conjugated Ab (1:2000, Dako A/S). Signals were visualized by enhanced chemiluminescence (Perkin-Elmer Life Science) and their densities were quantified by using ImageJ Version 1.32.

Indirect immunofluorescence microscopy. We obtained WT and mutant cDNA fragments of human *FHL2* by RT-PCR from human adult heart cDNA. Both cDNA fragments were cloned into pcDNA3.1/NT-GFP-TOPO (Invitrogen) and sequenced to confirm that no PCR errors were introduced. Each constructs was transfected into neonatal rat cardiomyocytes, C2C12, and RD21 cells. Cell culture, transfection procedures and fluorescence microscopy were performed as described previously [12].

Statistical analysis. Numerical data from M2H and IP assays were expressed arbitrarily as means ± standard deviation (SD). Statistical differences were examined by Student's *t*-test and *p* values of less than 0.05 were considered to be statistically significance.

Results

Identification of FHL2 mutation in DCM

Sequence variations in *FHL2* were searched for in DCM patients and six different variations were identified; two

variations in 5′ untranslated region, one non-synonymous change in exon 4 (c. 142G > A, p. Gly48Ser), one synonymous change in exon 7 and two synonymous changes in exon 8 (Fig. 1a). These variations other than Gly48Ser were found in healthy controls, suggesting that they were polymorphisms not related with DCM. In contrast, Gly48Ser (Fig. 1b) was identified in heterozygous state in a 49-years-old female patient who had apparent family history. This variation was not found in 576 control chromosomes, was located in the first LIM domain of FHL2 and replaced an evolutionary conserved residue in FHL subfamilies (Fig. 1c). These observations implied that it might be a DCM-associated mutation. Her mother was affected, but it was not clear whether her mother had the mutation or not, because she denied genetic testing.

Functional alterations in binding of FHL2 and titin/connectin caused by Gly48Ser mutation

To explore functional alterations caused by the *FHL2* mutation, we used mammalian-two-hybrid (M2H) assay and immunoprecipitation pull-down (IP) assay for binding of FHL2 with the N2B and is2 regions of titin/connectin. In the M2H assay, a bait plasmid containing wild-type (WT) or mutant *FHL2* cDNA was co-transfected with a prey plasmid containing *TTN* cDNA corresponding to

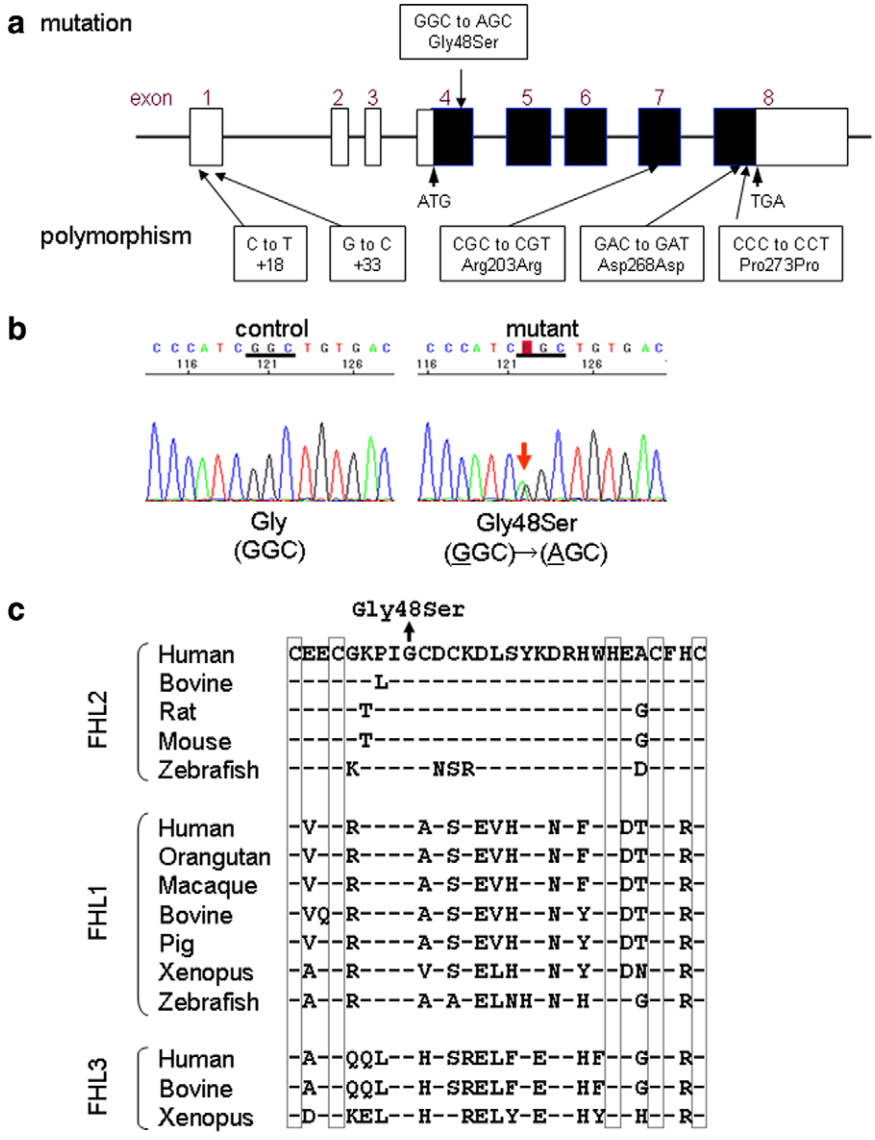

Fig. 1. Mutational analysis of *FHL2* in DCM. (a) Sequence variations found in this study are listed. Solid boxes represent protein coding region corresponding to exons 4–8. (b) Direct sequencing data of *FHL2* exon 4 from a control (left) and the DCM patient (right). Codon 48 of the control was GGC (Gly), whereas the patient was heterozygous for an AGC (Ser) mutation. (c) Protein sequence of human FHL2 predicted from nucleotide sequence was aligned with that of bovine, rat, mouse and zebrafish, along with FHL1 sequences from human, orangutan, macaque, bovine, pig, *Xenopus*, and zebrafish, and with FHL3 sequences from human, bovine and *Xenopus*. Cysteine and histidine residues highly conserved in the LIM domain are boxed. Dashes indicate identities to the human FHL2 sequence. The Gly48Ser mutation found in this study are indicated.

the N2B or is2 regions of titin/connectin and luciferase activity in the transfectants was measured. The transfectants containing either *FHL2* or *TTN* constructs alone showed negligible luciferase activity, indicating no self-activation in these constructs. The luciferase activity in the transfectants of mutant *FHL2* and WT *TTN*-N2B constructs (0.035 ± 0.001 AU) was significantly lower than that of WT *FHL2* and WT *TTN*-N2B constructs (0.127 ± 0.019 AU, $p < 0.001$). The transfectants were also investigated for the binding of FHL2 with titin/connectin by using the IP assay. Western blot analysis of immunoprecipitates from transfectants of WT or mutant *FHL2* with WT *TTN*-N2B revealed that, despite equal expression of genes, the mutant *FHL2* bound to *TTN*-N2B significantly less than WT *FHL2* (4353 ± 4934 vs. 12175 ± 2119 AU, $p < 0.05$) (Fig. 2a).

As for the binding between FHL2 and the is2 region of titin/connectin, the luciferase activity in the transfectants containing mutant *FHL2* and WT *TTN*-is2 constructs (0.025 ± 0.004 AU) was significantly lower than that of WT *FHL2* and WT *TTN*-is2 constructs (0.052 ± 0.014 AU, $p < 0.05$) . The lower binding of FHL2 with titin/connectin is2 region was also demonstrated by using the IP assay (2051 ± 533 AU vs. 9483 ± 1149 AU, $p < 0.001$) (Fig. 2b).

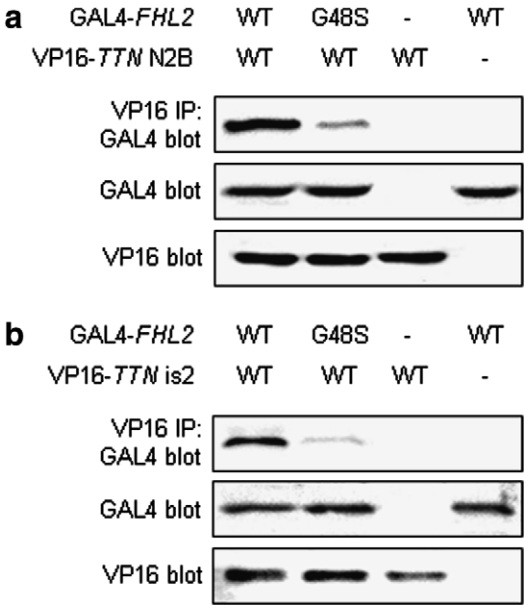

Fig. 2. Binding of FHL2 to titin/connectin. Binding of FHL2 to N2B region. (a) or is2 region (b) of titin/connectin was analyzed by IP using an anti-VP16 antibody. (a) GAL4-FHL2 co-precipitated with VP16-TTN N2B was detected by immunoblot using an anti-GAL4 antibody (top panel). Expression of GAL4-FHL2 (middle panel) and (lower panel) were confirmed by immunoblot of whole cell supernatants. Dashes indicate no GAL4 or VP16 proteins (transfected only with pBIND or pACT vectors, respectively). (b) GAL4-FHL2 co-precipitated with VP16-TTN is2 was shown (top panel). Expressions of GAL4-FHL2 (middle panel) and VP16-TTN is2 (lower panel) were confirmed by immunoblot of whole cell supernatants.

Cellular localization of FHL2

Since FHL2 is known to localize in Z-disc as well as focal contacts, we examined cellular localization of WT or mutant FHL2 being expressed in primary culture of rat cardiomyocytes or cell lines of muscle origin, C2C12 and RD21. We transfected the cells with GFP fusion constructs of WT or mutant FHL2 and examined GFP signals under confocal lasar microscopy. It was demonstrated that the WT GFP-FHL2 was mainly targeted to Z-discs in rat cardiomyocytes (Fig. 3a) and focal contacts in cell lines (Fig. 3c and e) and no apparent changes in localization of mutant GFP-FHL2 were observed (Fig. 3b, d, and f), suggesting that the overexpression of mutant FHL2 was neither deleterious to maintaining sarcomere structure nor causative for drastic change in cellular localization of FHL2.

Discussion

FHL2 is a member of heart-specific four and a half LIM-only proteins [16] and the Gly48Ser mutation is located at the ninth position next to a constant isoleucine within the first LIM domain. The LIM domain consists of 50–60 amino acid residues with a consensus sequence (Cys-X_2-Cys-X_3-Ile-X_{11-18}-Trp-His-X_2-Cys)-Phe-X-(Cys-X_2-Cys-X_3-Ile/Leu-X_4-Phe/Tyr-X_{8-15}-Cys-X_2-Cys/Asp/His) (where X represents any amino acids) and found in various proteins [23]. Physiochemical and structural analyses have revealed that the LIM domain is composed of two zinc-coordinated motifs which participate in a wide range of protein–protein interaction, regulating the function of cytoskeletal proteins, enzymes, or transcription factors [23,24]. It have been reported that several LIM proteins localize in the muscle sarcomere and knock-out mice of the sarcomere LIM proteins such as muscle LIM protein (MLP) [25] and actinin-associated LIM protein [26] displayed DCM phenotypes. In addition, we have identified DCM-associated mutations in other LIM proteins, MLP and Cypher/ZASP, which impaired protein-protein interaction; i.e., binding to Tcap and protein kinase C, respectively [11,21]. These observations suggest that the protein–protein interaction via LIM domain in components of cardiac sarcomere is important for cardiac function and its impairment would lead to DCM.

Lange et al. suggested that FHL2 acted as specific adaptor protein to couple metabolic enzymes to site of high energy consumption in the cardiac sarcomere through the interaction with titin/connectin [20]. The *FHL2* mutation found in this study affected the binding to both N2B and is2 regions of titin/connectin, implying that the mutation lead to impaired recruitment of these metabolic enzymes to cardiac sarcomere and hence to cardiac failure. The role of titin/connectin in the I-band region is not only responsible for passive tension generation upon stretch of non-activated striated muscle but also serving as hotspots of interactions with signaling molecules [6]. The cardiac

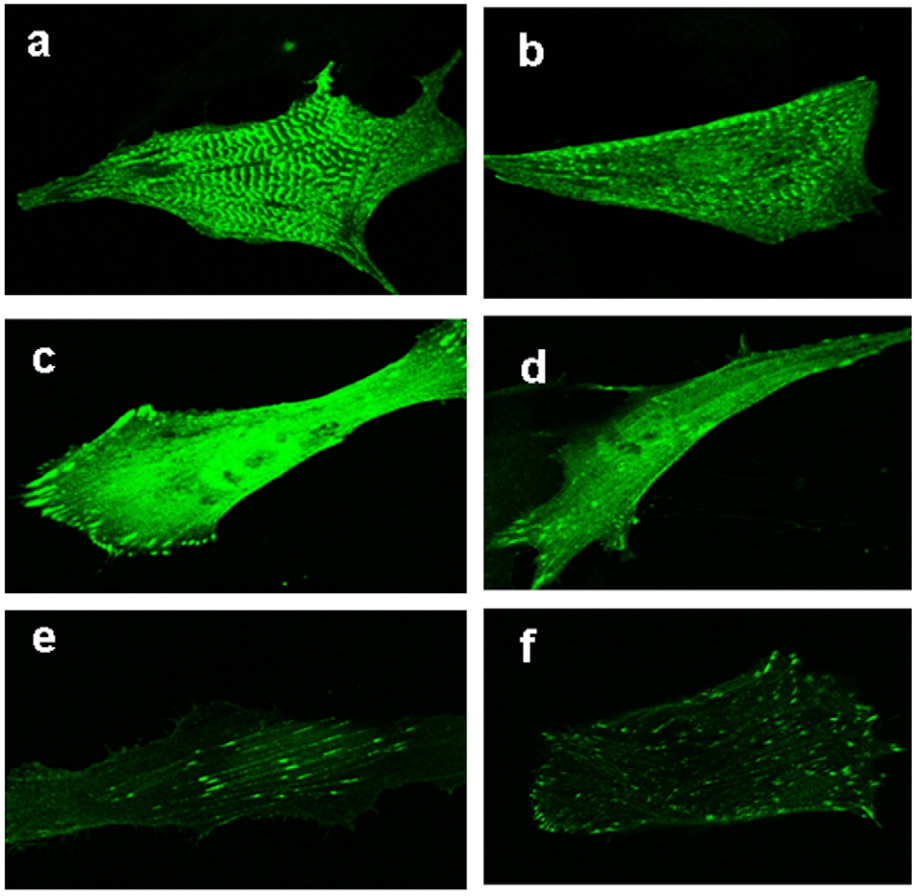

Fig. 3. Fluorescence images of transiently expressed WT and mutant FHL2 fused to GFP. WT or mutant FHL2 tagged with GFP were transfected to rat cardiomyocytes (a,b), C2C12 (c,d) or RD21 (e,f), and 24 h after the transfection the cells were processed for indirect immunofluorescence to detect the distribution of GFP signals. Localization of mutant FHL2-GFP (b, d, and f) was similar to that of WT FHL2-GFP (a, c, and e).

specific N2B domain is located in the central I-band region and plays a role as scaffold for localization of signaling proteins. We recently demonstrated that a DCM-associated *TTN* mutation in the N2B domain, Gln4053ter, affected binding of titin/connectin to FHL2 and αB-crystallin that is a chaperone belonging to small heat shock protein family [12,14]. In addition, an αB-crystallin mutation found in DCM also affected binding of αB-crystallin to titin/connectin [12]. These observations and the findings in this study strongly suggested that the abrogated interaction of titin/connectin N2B domain with the signaling molecules localized to the I-band region might cause cardiac dysfunction. The importance of N2B domain was also supported by the observation that overexpression of N-terminus to N2B domain of titin/connectin greatly disrupted thin filament in cardiomyocytes [27]. Studies on the function of titin/connectin N2B domain with N2B-associated proteins and functional alterations due to the DCM-causing mutations will help us to further understand the pathogenesis of DCM.

In summary, we identified an *FHL2* mutation associated with DCM. Because the mutation abrogated the interaction with titin/connectin, it was suggested that the abnormal recruitment of metabolic enzymes to cardiac sarcomere would cause DCM. Although the molecular mechanisms of DCM due to the *FHL2* mutation remain to be elucidated, our observations imply that the cardiac dysfunction and heart failure might be associated not only with the alteration of mechanical stretch response in each sarcomere components but also with the impairment/perturbation of the interaction between sarcomere and stretch-dependent signaling molecules.

Acknowledgments

We are grateful Drs. H. Toshima, H. Nishi, K. Matsuyama, H. Kagiyama, 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 clinical evaluation and blood sampling from patients with DCM. We also thank Dr. M. Yanokura and Ms. M. Emura for their technical assistance. This work was supported in part by Grant-in-aids from the Ministry of Education, Culture, Sports, Science and technology, Japan, research grants from the Ministry of Health, Labour and Welfare, Japan, the Program for Promotion of Fundamental Studies in Health

Sciences of the National Institute of Biomedical Innovation (NIBIO), Mitsubishi Pharma Research Foundation, Miyata Foundation for cardiac disease research, and ''Association Françaiise contre les Myopathies'' (AFM, Grant Nos. 11057 and 11737).

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] V.V. Michels, P.P. Moll, F.A. Miller, A.J. Tajik, J.S. Chu, D.J. Driscoll, J.C. Burnett, R.J. Rodeheffer, J.H. Chesebro, H.D. Tazelaar, The frequency of familial dilated cardiomyopathy in a series of patients with idiopathic dilated cardiomyopathy, N. Engl. J. Med. 326 (1992) 77–82.

[3] 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.

[4] H. Morita, J. Seidman, C.E. Seidman, Genetic causes of human heart failure, J. Clin. Invest. 115 (2005) 518–526.

[5] L. Tskhovrebova, J. Trinick, Titin: properties and family relationships, Nat. Rev. Mol. Cell Biol. 4 (2003) 679–689.

[6] H.L. Granzier, S. Labeit, The giant protein titin: a major player in myocardial mechanics, signaling, and disease, Circ. Res. 94 (2004) 284–295.

[7] E. Flashman, C. Redwood, J. Moolman-Smook, H. Watkins, Cardiac myosin binding protein C: its role in physiology and disease, Circ. Res. 94 (2004) 1279–1289.

[8] S. Daehmlow, J. Erdmann, T. Knueppel, C. Gille, C. Froemmel, M. Hummel, R. Hetzer, V. Regitz-Zagrosek, Novel mutations in sarcomeric protein genes in dilated cardiomyopathy, Biochem. Biophys. Res. Commun. 298 (2002) 116–120.

[9] T.M. Olson, V.V. Michels, S.N. Thibodeau, Y.S. Tai, M.T. Keating, Actin mutations in dilated cardiomyopathy, a heritable form of heart failure, Science 280 (1998) 750–752.

[10] B. Mohapatra, S. Jimenez, J.H. Lin, K.R. Bowles, K.J. Coveler, J.G. Marx, M.A. Chrisco, R.T. Murphy, P.R. Lurie, R.J. Schwartz, P.M. Elliott, M. Vatta, W. McKenna, J.A. Towbin, N.E. Bowles, Mutations in the muscle LIM protein and alpha-actinin-2 genes in dilated cardiomyopathy and endocardial fibroelastosis, Mol. Genet. Metab. 80 (2003) 207–215.

[11] T. Hayashi, T. Arimura, M. Itoh-Satoh, K. Ueda, S. Hohda, N. Inagaki, M. Takahashi, H. Hori, M. Yasunami, H. Nishi, Y. Koga, H. Nakamura, M. Matsuzaki, B.Y. Choi, S.W. Bae, C.W. You, K.H. Han, J.E. Park, R. Knoll, M. Hoshijima, K.R. Chien, A. Kimura, Tcap gene mutations in hypertrophic cardiomyopathy and dilated cardiomyopathy, J. Am. Coll. Cardiol. 44 (2004) 2192–2201.

[12] N. Inagaki, T. Hayashi, T. Arimura, Y. Koga, M. Takahashi, H. Shibata, K. Teraoka, T. Chikamori, A. Yamashina, A. Kimura, Alpha B-crystallin mutation in dilated cardiomyopathy, Biochem. Biophys. Res. Commun. 342 (2006) 379–386.

[13] 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.

[14] Y. Matsumoto, T. Hayashi, N. Inagaki, M. Takahashi, S. Hiroi, T. Nakamura, T. Arimura, K. Nakamura, N. Ashizawa, M. Yasunami, T. Ohe, K. Yano, A. Kimura, Functional analysis of titin/connectin N2-B mutations found in cardiomyopathy, J. Muscle Res. Cell Motil. 26 (2005) 367–374.

[15] M. Genini, P. Schwalbe, F.A. Scholl, A. Remppis, M.G. Mattei, B.W. Schafer, Subtractive cloning and characterization of DRAL, a novel LIM-domain protein down-regulated in rhabdomyosarcoma, DNA Cell Biol. 16 (1997) 433–442.

[16] K.K. Chan, S.K. Tsui, S.M. Lee, S.C. Luk, C.C. Liew, K.P. Fung, M.M. Waye, C.Y. Lee, Molecular cloning and characterization of FHL2, a novel LIM domain protein preferentially expressed in human heart, Gene 210 (1998) 345–350.

[17] M. Johannessen, S. Moller, T. Hansen, U. Moens, M. Van Ghelue, The multifunctional roles of the four-and-a-half-LIM only protein FHL2, Cell Mol. Life Sci. 63 (2006) 268–284.

[18] N.H. Purcell, D. Darwis, O.F. Bueno, J.M. Muller, R. Schule, J.D. Molkentin, Extracellular signal-regulated kinase 2 interacts with and is negatively regulated by the LIM-only protein FHL2 in cardiomyocytes, Mol. Cell Biol. 24 (2004) 1081–1095.

[19] Y. Kong, J.M. Shelton, B. Rothermel, X. Li, J.A. Richardson, R. Bassel-Duby, R.S. Williams, Cardiac-specific LIM protein FHL2 modifies the hypertrophic response to beta-adrenergic stimulation, Circulation 103 (2001) 2731–2738.

[20] S. Lange, D. Auerbach, P. McLoughlin, E. Perriard, B.W. Schafer, J.C. Perriard, E. Ehler, Subcellular targeting of metabolic enzymes to titin in heart muscle may be mediated by DRAL/FHL-2, J. Cell Sci. 115 (2002) 4925–4936.

[21] T. Arimura, T. Hayashi, H. Terada, S.Y. Lee, Q. Zhou, M. Takahashi, K. Ueda, T. Nouchi, S. Hohda, M. Shibutani, M. Hirose, J. Chen, J.E. Park, M. Yasunami, H. Hayashi, A. Kimura, A Cypher/ZASP mutation associated with dilated cardiomyopathy alters the binding affinity to protein kinase C, J. Biol. Chem. 279 (2004) 6746–6752.

[22] A. Kimura, H. Harada, J.E. Park, H. Nishi, M. Satoh, M. Takahashi, S. Hiroi, T. Sasaoka, N. Ohbuchi, T. Nakamura, T. Koyanagi, T.H. Hwang, J.A. Choo, K.S. Chung, A. Hasegawa, R. Nagai, O. Okazaki, H. Nakamura, M. Matsuzaki, T. Sakamoto, H. Toshima, Y. Koga, T. Imaizumi, T. Sasazuki, Mutations in the cardiac troponin I gene associated with hypertrophic cardiomyopathy, Nat. Genet. 16 (1997) 379–382.

[23] I. Sanchez-Garcia, T.H. Rabbitts, The LIM domain: a new structural motif found in zinc-finger-like proteins, Trends Genet. 10 (1994) 315–320.

[24] G.C. Perez-Alvarado, C. Miles, J.W. Michelsen, H.A. Louis, D.R. Winge, M.C. Beckerle, M.F. Summers, Structure of the carboxy-terminal LIM domain from the cysteine rich protein CRP, Nat. Struct. Biol. 1 (1994) 388–398.

[25] S. Arber, J.J. Hunter, J. Ross Jr., M. Hongo, G. Sansig, J. Borg, J.C. Perriard, K.R. Chien, P. Caroni, MLP-deficient mice exhibit a disruption of cardiac cytoarchitectural organization, dilated cardiomyopathy, and heart failure, Cell 88 (1997) 393–403.

[26] M. Pashmforoush, P. Pomies, K.L. Peterson, S. Kubalak, J. Ross Jr., A. Hefti, U. Aebi, M.C. Beckerle, K.R. Chien, Adult mice deficient in actinin-associated LIM-domain protein reveal a developmental pathway for right ventricular cardiomyopathy, Nat. Med. 7 (2001) 591–597.

[27] W.A. Linke, D.E. Rudy, T. Centner, M. Gautel, C. Witt, S. Labeit, C.C. Gregorio, I-band titin in cardiac muscle is a three-element molecular spring and is critical for maintaining thin filament structure, J. Cell Biol. 146 (1999) 631–644.