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REVIEW

Molecular genetics and pathogenesis of cardiomyopathy

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Cardiomyopathy is defined as a disease of functional impairment in the cardiac muscle and its etiology includes both extrinsic and intrinsic factors. Cardiomyopathy caused by the intrinsic factors is called as primary cardiomyopathy of which two major clinical phenotypes are hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM). Genetic approaches have revealed the disease genes for hereditary primary cardiomyopathy and functional studies have demonstrated that characteristic functional alterations induced by the disease-associated mutations are closely related to the clinical types, such that increased and decreased Ca^{2+} sensitivities of muscle contraction are associated with HCM and DCM, respectively. In addition, recent studies have suggested that mutations in the Z-disc components found in HCM and DCM may result in increased and decreased stiffness of sarcomere, respectively. Moreover, functional analysis of mutations in the other components of cardiac muscle have suggested that the altered response to metabolic stresses is associated with cardiomyopathy, further indicating the heterogeneity in the etiology and pathogenesis of cardiomyopathy.

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INTRODUCTION

Cardiomyopathy is a heterogeneous disease caused by functional abnormality of cardiac muscle and classified into primary cardiomyopathy and secondary cardiomyopathy.[1] Secondary cardiomyopathy is defined as cardiomyopathy caused by extrinsic factors including ischemia, hypertension and metabolic disorders. On the other hand, diagnosis of primary cardiomyopathy is based on the exclusion of secondary cardiomyopathy and there are several different clinical types.[2,3] Hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) are two major clinical types of primary cardiomyopathy. HCM, a major cause of sudden death in young and heart failure, is characterized by left ventricular hypertrophy, often asymmetric, accompanied by myofibrillar disarrays and diastolic dysfunction of cardiac ventricles. In contrast, DCM is characterized by dilated ventricular cavity with systolic dysfunction. Clinical symptom of DCM is heart failure and often associated with sudden death. In addition, there are other clinical types of cardiomyopathy. Restrictive cardiomyopathy (RCM) is accompanied by increased stiffness of the myocardium with diastolic dysfunction without significant hypertrophy,[4] and arrhythmogenic right ventricular cardiomyopathy (ARVC) is characterized by a dilated dysfunctional right ventricle (RV), ventricular arrhythmias and fibrofatty replacement of the RV.[1] Another cardiomyopathy is left ventricular noncompaction (LVNC) characterized by trabeculations in the left ventricle (LV), as well as LV hypertrophy and/or dilation.[1]

The etiology of primary cardiomyopathy had been unknown, but various genetic abnormalities associated with the cardiomyopathy have recently been unraveled. More than half of HCM patients have family history of the disease consistent with autosomal dominant genetic trait.[5] In the case of DCM, about 20–35% patients had family history of the disease, mainly consistent with the autosomal dominant inheritance, although some familial cases can be explained by autosomal recessive or X-linked recessive trait.[6,7] Because the presence of family history suggests the genetic etiology, linkage studies have been performed to identify the disease loci in each multiplex family with cardiomyopathy. Identification of the disease loci has enabled to decipher the disease-linked mutations in the genes located within the loci. Subsequently, candidate gene approaches, focused on the genes encoding for proteins related or interacting with products of the previously identified disease genes, have been successful in unraveling novel disease genes. As shown in Table 1, many different disease genes have so far been identified. The most important issue is the overlapping of disease genes for different clinical types.

MUTATIONS IN GENES FOR CONTRACTILE ELEMENTS IN CARDIOMYOPATHY

Identification of a missense mutation in cardiac β-myosin heavy chain gene (*MYH7*) linked to HCM in a large multiplex family was the first demonstration of the disease gene for HCM.[8] At the same time an accompanying paper reported a fusion gene of *MYH7* and cardiac α-myosin heavy chain gene (*MYH6*) in another multiplex family.[9] It was later revealed that there was a linked *MYH7* missense mutation[10] in the family with the *MYH6-MYH7* fusion gene, suggesting that *MYH7* mutations are the major cause of HCM. Many

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Table 1 Disease genes for hereditary cardiomyopathy

Clinica phenotype	*Heredity*	*Gene symbol*	*Coding protein*
HCM/DCM/RCM/LVNC	AD	*MYH7*	Cardiac β-myosin heavy chain
HCM/DCM/RCM/LVNC	AD	*TNNT2*	Cardiac troponin T
HCM/DCM/RCM/LVNC	AD	*TPM1*	α-Tropomyosin
HCM/DCM/LVNC	AD	*MYBPC3*	Cardiac myosin binding protein-C
HCM/RCM	AD	*MYL3*	Ventricular myosin essential light chain
HCM/RCM	AD	*MYL2*	Ventricular myosin regulatory light chain
HCM/DCM/RCM	AD	*TNNI3*	Cardiac troponin I
HCM/DCM/LVNC	AD	*ACTC1*	Cardiac α-actin
HCM/DCM/ARVC	AD	*TTN*	Titin, connectin
HCM/DCM	AD	*TNNC1*	Cardiac troponin C
HCM/DCM	AD	*MYH6*	Cardiac α-myosin heavy chain
HCM/DCM	AD	*CSRP3*	Muscle LIM protein, MLP
HCM	AD	*CAV3*	Caveolin-3
HCM/DCM	AD	*TCAP*	Titin-cap, Tcap, telethonin
HCM/DCM	AD	*VCL*	Metavinculin
HCM	AD	*JPH2*	Junctophilin-2
HCM	AD	*OBSCN*	Obscurin
HCM	AD	*MYOZ2*	Myozenin, calsartin-1
HCM/DCM	AD	*ANKRD1*	CARP
HCM/DCM	AD	*RAF1*	RAF1 (MAP3K)
HCM	XD	*FHL1*	Four and a half LIM protein-1, FHL1
DCM/RCM	AD	*DES*	Desmin
DCM/LVNC	AD	*LMNA*	Lamin A/C
DCM	AD	*SAGD*	δ-Sarcoglycan
DCM	AD	*ACTN2*	α-Actinin-2
DCM/LVNC	AD	*LDB3*	Cypher, ZASP, oracle
DCM/HCM	AD	*PLN*	Phospholamban
DCM	AD	*ABCC9*	K_{ATP} channel
DCM	AD	*SCN5A*	Cardiac Na channel
DCM/HCM	AD	*CRYAB*	αB crystallin
DCM	AD	*PSEN1*	Presenilin-1
DCM	AD	*PSEN2*	Presenilin-2
DCM	AD	*FHL2*	Four and a half LIM protein-2, FHL2
DCM	AD	*LMNA4*	Laminin α4
DCM	AD	*ILK*	Integrin-linked kinase
DCM/HCM/RCM	AD	*MYPN*	Myopalladin
DCM	AD	*RBM20*	RNA binding motif protein 20
DCM/HCM	AD	*NEXN*	Nexillin
DCM/EFE	AD	*NEBL*	Nebulette
DCM	AD	*BAG3*	Bcl2-associated athanogene protein
DCM	AD	*MURC*	Muscle-related coiled coil protein
DCM	AD	*FHOD3*	Formin homology 2 domain containing 3
DCM	AD	*TNNI3K*	Troponin I kinase
DCM	AD	*CHRM2*	Acetylcholine receptor
DCM	AR	*FKTN*	Fuktin
DCM	AR	*GATAD1*	GATAD1
DCM	AR	*SDHA*	Succinate dehydrogenase complex, subunit A
DCM	AR	*DOLK*	Dolichol kinase
DCM	XR	*DMD*	Dystrophin
DCM	XR	*EMD*	Emerin
LVNC/DCM	XR	*TAZ*	Tafazzin, G4.5
DCM	XR	*FKTN*	Fuktin
ARVC/DCM/ARVC	AR	*DSP*	Desmoplakin
ARVC/DCM	AR, AD	*JUP*	Plakoglobin
ARVC	AD	*PKP2*	Plakophilin-2
ARVC	AD	*TGFB3*	TGFβ3
ARVC	AD	*RYR2*	Ryanodine receptor 2
ARVC	AD	*DSG3*	Desmoglein 3
ARVC	AD	*MIB1*	E3 ubiquitin ligase
LVNC	AD	*DTNA*	α-Dystrobrebin

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; ARVC, arrhythmogenic right ventricular cardiomyopathy; DCM, dilated cardiomyopathy; EFE, endocardial fibroelastosis; HCM, hypertrophic cardiomyopathy; LVNC, left ventricular noncompaction; RCM, restrictive cardiomyopathy; XD, X-linked dominant; XR, X-linked recessive.

investigators have analyzed HCM patients for mutations in *MYH7* and many different missense mutations were identified,[10] but the frequency of *MYH7* mutations in the HCM patients was less than half and there were many families not linked to the *MYH7* locus.[10–13] Linkage studies in such non-*MYH7*-linked HCM families have revealed mutations in α-tropomyosin gene (*TPM1*), cardiac troponin T gene (*TNNT2*) and cardiac myosin binding protein-C gene (*MYBP3*) as the causes of HCM. Because these disease genes encode contractile elements of sarcomere, genes for other contractile components were analyzed and lead to the identification of HCM-associated mutations in ventricular myosin essential light chain gene (*MYL3*), ventricular myosin regulatory light chain gene (*MYL2*), cardiac troponin I gene (*TNNI3*), cardiac α-actin gene (*ACTC1*) and cardiac troponin C gene (*TNNC1*).[3,11–13]

We have reported that contractile element mutations are found in about 40% of Japanese patients with familial HCM in the heterozygous state, consistent with the autosomal dominant inheritance, and about 17%, 14% and 11% of HCM patients carried mutations in *MYH7*, *MYBPC3* and *TNNT2*, respectively, while a few cases had mutations in other component genes such as *MYL2*, *MYL3* and *TNNI3* (Table 2). A systematic survey of mutations in consecutive 112 Japanese familial HCM patients showed that a few patients (two cases, 1.8%) had mutations in two different disease genes (double mutation case).[14] The double mutation cases developed cardiac hypertrophy earlier than the single mutation case in the same family.[14] In addition, we also reported that homozygous patients showed severer clinical manifestations than heterozygous patients in the same family, demonstrating the gene dose effect of mutation.[15] Disease-related mutations can also be found in sporadic cases who had no family history (Table 2). Mutations in sporadic cases included *de novo* mutation cases,[16,17] but the other sporadic cases were probably due to the low penetrance of mutation, because most of the mutations found in the sporadic HCM patients were found in other familial HCM patients.

Mutations in genes for contractile elements have also been found in DCM patients. Identification of cardiac α-actin gene (*ACTC1*) mutations was the first discovery of genetic cause of autosomal dominant DCM.[18] Subsequently, *ACTC1* mutation was found in HCM,[19] first demonstration of an overlapping of disease genes for different primary cardiomyopathies. Molecular basis of different phenotypes caused by *ACTC1* mutations was explained by the difference in which functional domains were affected; DCM-associated mutations were found at the α-actinin interacting domain,[18] whereas HCM-associated mutations were at the interacting domain to myosin heavy chain.[19] On the other hand, recent data

Table 2 Frequencies of disease-associated mutations in Japanese HCM

a: Mutations in genes for contractile elements

Gene symbol	%Familial case (n = 282)	%Sporadic case (n = 100)
MYH7	17.2	2.0
TNNT2	10.6	3.0
TPM1	1.8	0.0
MYBPC3	14.2	5.0
MYL3	0.4	1.0
MYL2	0.7	0.0
TNNI3	2.1	3.0
ACTC1	0.0	0.0
Sum	46.8	14.0

b: Mutations in other genes

Gene symbol	%Familial case (n = 162)	%Sporadic case (n = 100)
TTN[a]	>3.7	>2.0
CSRP3	0.0	0.0
TNNC1	0.0	0.0
CAV3	0.6	0.0
TCAP	1.2	0.0
CRYAB	0.0	0.0
VCL	0.0	0.0
OBSCN	0.6	0.0
ANKRD1	0.6	0.0
Sum	>6.2	>2.0

[a]Z-disc, N2-B, N2-A, titn-kinase, Novex3 and is2 domains (about 25% of entire *TTN*) were analyzed.

Table 3 Frequencies of disease-associated mutations in Japanese DCM

Gene symbol	%Familial case (n = 48)	%Sporadic case (n = 100)
ACTC1	0.0	0.0
DES	2.1	0.0
DMD	0.0	5.0
LMNA	0.0	nt
SAGD	0.0	nt
MYH7	8.7	0.0
TNNT2	0.0	0.0
TPM1	0.0	0.0
TTN[a]	>6.3	>2.0
CSRP3	0.0	0.0
VCL	0.0	0.0
CRYAB	2.1	0.0
MYBPC3	0.0	0.0
TCAP	2.1	0.0
ACTN2	0.0	0.0
LDB3	2.1	0.0
FKTN	0.0	0.0
FHL2	2.1	0.0
MYPN	0.0	0.0
RBM20	0.0	0.0
BAG3	4.2	0.0
LMNA4	0.0	0.0
ILK	0.0	0.0
FHOD3	2.1	0.0
ANKRD1	0.0	0.0
Sum	>31.3	>7.0

Abbreviation: nt, not tested.
[a]Z-disc, N2-B, N2-A, titn-kinase, Novex3 and is2 domains (about 25% of entire *TTN*) were analyzed.

suggest that there is a difference in folding property of actin between the DCM-associated mutation and HCM-associated mutation.[20] Another example of overlapping disease gene was the identification of *TNNT2* mutation in DCM.[21] As discussed later, functional studies of *TNNT2* mutations clearly demonstrated the difference between the DCM-associated mutation and HCM-associated mutation.[22,23] Therefore, contractile element mutations can be found in both HCM and DCM, but difference in the functional alterations may determine the different phenotypes.[24] However, as shown in Table 3, frequencies of contractile element abnormalities in DCM patients were considerably small as compared with those in HCM patients.

Initial analysis of functional changes caused by the *MYH7* mutations demonstrated that contractile power generation was decreased in the presence of mutant myosin heavy chains[25] and HCM-related mutations were found in other contractile elements, troponin T and α-tropomyosin. From these observations, it was hypothesized that HCM was the disease of sarcomere and the cardiac hypertrophy was a compensation of decreased contractility of cardiac muscle.[26] However, the discovery of HCM-associated *TNNI3* mutations at the contraction inhibitory domain[16] implied that the decreased power might not be a common functional change caused by the contractile element mutations. Indeed, subsequent functional analyses of gene mutations in other contractile elements than *MYH7* have revealed that most of the HCM-associated mutations resulted in an increased Ca^{2+} sensitivity of muscle contraction.[23,27–31] Because a *MYH7* mutation that caused HCM in transgenic mice also increased Ca^{2+} sensitivity at the muscle fiber level,[32] a common functional alteration due to the HCM-related sarcomere mutations may be the increased Ca^{2+} sensitivity. Muscle contraction is regulated by the concentration of intracellular Ca^{2+} that is released from sarcoplasmic reticulum via ryanodine receptor (RyR2) and restored to sarcoplasmic reticulum via sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA). When the concentration of Ca^{2+} is increased or decreased, muscle is contracted or relaxed, respectively. In other words, the increased Ca^{2+} sensitivity means a leftward shift of Ca^{2+}-tension curve; more tension is generated by mutant contractile elements than normal elements at the same Ca^{2+} concentration (hyper-contraction) or muscle with mutant elements is under less relax states (diastolic dysfunction) than the normal muscles. This is consistent with the finding that characteristic features of HCM are hyper-contraction and diastolic dysfunction.

On the other hand, it was demonstrated in model mice carrying cardiomyopathy-associated *TNNT2* mutations that Ca^{2+} sensitivity was increased by a HCM-associated delGlu160 mutation, whereas it was decreased by a DCM-associated delLys210 mutation.[22] These observations indicated that DCM-associated contractile element mutations lead to systolic dysfunction of the heart. It should be noted here that drugs or chemicals that increased Ca^{2+} sensitivity could be effective in preventing heart failure in the mouse models carrying the DCM-associated *TNNT2* mutation.[33]

MUTATIONS IN GENES FOR Z-DISC ELEMENTS IN CARDIOMYOPATHY

Because mutations in the contractile elements were found in only less than half of HCM patients and only in a few DCM cases, there should

be other disease gene(s) for HCM and DCM so that candidate gene approaches were taken to identify the disease-related mutations in other genes expressed in cardiac muscle (Figure 1). Identification of a HCM-associated mutation in titin gene (*TTN*) was the first example of disease gene other than the contractile elements,[34] and the functional alteration due to the *TTN* mutation was an increased binding to α-actinin[34]. In addition, it was demonstrated that the HCM-associated Tcap gene (*TCAP*) mutations increased the binding of Tcap to titin, MLP and carsarcin-1,[35] leading to a hypothesis that Z-disc mutations in HCM may result in increased binding of Z-disc components rendering sarcomere stiff. 'Stiff sarcomere' would increase passive tension upon stretch of sarcomere. Because the increased passive tension was associated with an increased Ca^{2+} sensitivity,[36–38] it was speculated that HCM-associated abnormality in both Z-disc components and contractile elements might commonly result in the increased Ca^{2+} sensitivity of cardiac muscle. It should be noted that a possible controversy exists, that is, HCM-associated MLP gene (*CSRP3*) mutations were reported to decrease the binding to α-actinin and N-RAP.[39,40] However, DCM-associated mutations were also found in *CSRP3* and α-actinin gene (*ACTN2*) and these mutations decreased binding to each other.[41] Therefore, the decreased binding between MLP and α-actinin was associated with both HCM and DCM. This discrepancy should be resolved by further studies.

Mutations in Z-disc components were relatively frequent in DCM patients (Table 3). We have reported several DCM-associated Z-disc component mutations in *TTN*,[42,43] *CSRP3*,[44] *TCAP*[35,44] and Cypher/ZASP gene (*LDB3*).[45] It was demonstrated that the DCM-associated *TCAP* mutations showed opposite functional alterations to the HCM-associated mutations.[35] Similarly, a DCM-associated *TTN* mutation found in the actinin-binding domain showed a decreased binding to actinin.[42] In addition, another DCM-associated *TTN* mutation found in the Tcap binding domain decreased the binding to Tcap.[42] Because the Z-disc element mutations result in decreased binding among the elements, we hypothesize that DCM is the disease of 'loose sarcomere'.[12,35] The loose sarcomere is evident in an animal model of DCM, a *CSRP3* (MLP) knock-out mouse, in which Z-disc was wide and stretch response was impaired.[44] Because the stretch response is a hypertrophic response of cardiomyocytes against passive tension and Z-disc elements have a role of stretch sensor in cardiomyocytes, abnormality in the Z-disc elements may alter the regulation of stretch response. In addition, molecular mechanisms for developing DCM due to the *CSRP3* mutation[44] were investigated in a knock-in mouse model.[45]

Cypher/ZASP is a Z-disc element connecting calsarcin and actinin.[46] Calsarcin binds calcineurin,[47] a Ser/Thr phosphatase involved in the process of hypertrophic program of cardiomyocytes.[48] Functional significance of calcineurin anchorage to Z-disc is not fully understood, but it is involved in stress-induced calcineurin-NFAT activation, because heterozygous *CSRP3* knock-out mice showed reduction in NFAT activation along with a dislocation of calcineurin from Z-disk.[49] On the other hand, Cypher/ZASP binds protein kinase C[46] and a DCM-associated *LDB3* mutation in the protein kinase C-binding domain was found to increase the binding,[50] suggesting that phosphorylation/dephosphorylation of Z-disc elements might be involved in the stretch response. Several other *LDB3* mutations not in the protein kinase C-interacting domain were reported in DCM or LVNC.[51] We searched for a binding protein to Cypher/ZASP by using the yeast two-hybrid method, and found phosphoglucomutase-1 (PGM1) as a novel binding protein.[52] PGM1 is an enzyme involved in the glucose/glycogen metabolism and we demonstrated that PGM1 was localized at the Z-disc under the stressed culture conditions, low serum and low glucose, suggesting the role of PGM1 in the energy metabolism at the Z-disc.[52] Because the DCM-associated mutations decreased the binding to PGM1,[52] it was suggested that the decreased stress response might be involved in the pathogenesis of DCM.

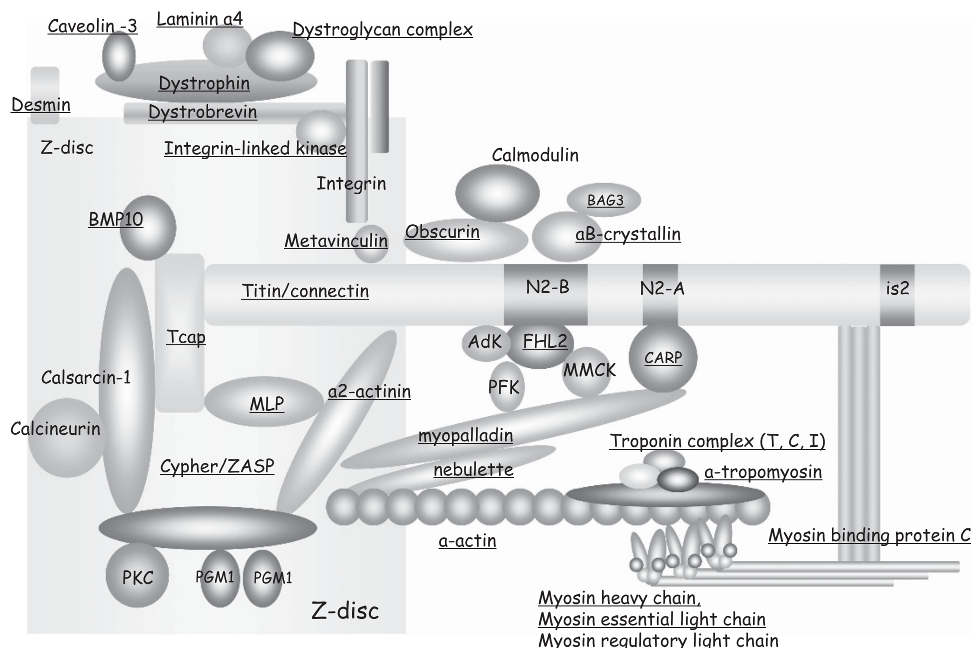

Figure 1 Schematic representation of sarcomere components. Half sarcomere is schematically shown. Components are underlined when disease-associated mutations have been identified in patients with primary cardiomyopathy. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

There are several other DCM-associated mutations found in genes for the other Z-disk associated proteins, desmin (*DES*)[53] and metavinculin (*VCL*).[54] The *VCL* mutation impaired the binding to actin,[54] while the *DES* mutations resulted in a subtle change in the cytoplasmic desmin network.[55] In addition, mutations in myopalladin gene (*MYPN*) have recently been reported in DCM. Although the molecular mechanisms of *MYPN* mutations leading to DCM remained unclear, the DCM-associated mutations impaired the myofiblinogenesis.[56]

TTN MUTATIONS IN CARDIOMYOPATHY

Titin is the most giant protein so far known and it spans from the Z-disc to M-line of sarcomere.[57] As noted above, cardiomyopathy-associated mutations were found in the Z-disc region of titin,[42] but a large body of DCM-associated mutations were found in other regions than the Z-disc region, that is, in the I-band region and M-band region. Initial reports of *TTN* mutations found in DCM patients included nonsense mutations and frameshift mutations[42,43] encoding truncated titin proteins. Recently, a systematic analysis of entire *TTN* coding exons has revealed that *TTN* is a major disease gene for DCM, because *TTN* mutations were found in about 26.7% of DCM patients; in addition to that; about 1.3% of HCM patients and 2.8% of healthy controls carried *TTN* mutations.[58] In addition, it was found that most of the *TTN* mutations found in DCM patients were suggested to encode truncated proteins, while those in HCM were missense mutations.[58] On the other hand, it was reported that *TTN* mutations could be found in many DCM families, but not all of them were co-segregated with the disease.[59] In addition, *TTN* mutations including nonsense and frameshift mutations could also be found in healthy individuals, raising a possibility that not all of the *TTN* mutations found in patients with cardiomyopathy were disease-causing mutations.[60] Recently, a large systematic analysis of *TTN* mutations has demonstrated that truncated mutations, that is, nonsense, frameshift and splicing site mutations, were found in both DCM patients and healthy controls, but the distribution of mutations was different between them. Mutations found in DCM patients were significantly enriched in the 3′ half of *TTN*, especially in the region encoding for A-band region.[61] It is therefore suggested that the rare variants of *TTN* in the A-band region may be genetic risk factors for heart failure and it is not clear enough at the moment whether the *TTN* rare variants by themselves could cause DCM as the monogenic disease gene mutations with low penetrance or they are involved in the pathogenesis of DCM as genetic risk factors for polygenic disease. Nevertheless, *TTN* truncation mutations could contribute to development of heart failure, suggesting that the analysis of *TTN* would be useful in predicting clinical courses of DCM patients.[61]

MEMBRANOUS AND CYTOSKELETAL MUTATIONS IN CARDIOMYOPATHY

Identification of mutation in dystrophin gene (*DMD*) in male siblings of X-linked DCM[62] was the first discovery of a disease gene for DCM. X-linked DCM is a rare form of familial DCM almost exclusively affecting males.[63] *DMD* mutations are known to cause muscular dystrophy, which mainly affects skeletal muscles, and cardiac involvement is usually observed later in the clinical course.[64,65] In contrast, X-linked DCM cases usually manifest with cardiac symptoms and subtle skeletal muscle involvement,[63] and phenotypic variance of *DMD* mutations may be explained by detecting which domain of dystrophin was affected.[64] As shown in Table 3, *DMD* mutations could be found in 5% of Japanese sporadic DCM cases. None of these patients showed skeletal muscle symptoms, demonstrating that X-linked DCM should be considered not only for the male sibling of familial DCM but also for male cases of sporadic DCM.

Dystrophin is a membranous protein having a key role in mechanical links from extracellular matrix to intracellular cytoskeleton in association with other proteins forming a dystroglycan complex (DGC).[66] Because muscle contraction forces deformity of myocytes with shortening/stretching, myofilaments should be tightly anchored to membrane and extracellular matrix via DGC to properly transmit the force with avoiding damages of cell membrane. Components of DGC in skeletal and cardiac muscles include dystrophin, α- and β-dystroglycans, α-laminins, α-, β-, γ- and δ-sarcoglycans, α- and β-dystrobrevins, syntrophin, and caveolin-3. In addition to DGC, α- and β-integrins are concentrated at the costameres that overly Z-lines in striated muscles, and the integrin complex also has a crucial role in mechanical links of power transmission.[66] Therefore, abnormalities in DGC and integrin complex may result in muscular dystrophy and cardiomyopathy. Indeed, mutations in δ-sarcoglycan gene (*SAGD*),[67] laminin α4 gene (*LMNA4*)[68] and integrin-linked kinase gene (*ILK*)[68] were found to cause DCM of autosomal dominant inheritance (Table 1).

OTHER MUTATIONS IN CARDIOMYOPATHY

There are several other disease genes for HCM, including mutations in caveolin-3 gene (*CAV3*),[69] meta-vinculin gene (*VCL*),[70] αB-crystallin gene (*CRYAB*),[71] junctophilin-2 gene (*JPH-2*),[72] obscurin gene (*OBSCN*)[73] and CARP gene (*ANKRD1*)[74] (Figure 1). Functional analyses were reported for *CRYAB*, *CAV3*, *OBSCN* and *ANKRD1* mutations; aggregation of αB-crystalline in cytoplasm,[71] decreased cell surface expression of caveolin-3,[69] decreased binding to titin[73] and increased binding to titin and myopalladin,[74] respectively. It is not clear how the aggregated αB-crystalline resulted in cardiac hypertrophy, but impaired stress response may exaggerate hypertrophic response.[75] It is interesting to note that an HCM-associated *TTN* mutation in N2B region increased binding to FHL2 protein[76] and decreased binding to αB-crystalline.[77] Although function of obscurin is not fully understood, it may be involved in a calmodulin/CaMK-mediated signaling, because obscurin was reported to tether calmodulin to titin,[78] which was impaired by the HCM-associated *OBSCN* mutation. The functional significance of increased binding of CARP to titin and myopalladin caused by the *ANKRD1* mutations is not fully clarified, but mutant CARPs showed nuclear or perinuclear localization, whereas normal CARP was exclusively localized in the cytoplasm.[79] Because CARP is a hypertrophy-related transcriptional co-factor[79] and is known to be localized in the cytoplasm but shifted to nucleus upon stretching cardiomyocytes,[80] abnormal nuclear localization of mutant CARPs suggested that the mutations rendered cardiomyocytes hypersensitive to the stretch response leading to hypertrophy. It was recently reported that three different *ANKRD1* (CARP) mutations, all of which increased binding to titin/myopalladin and localized to nuclei, showed different functional changes in reduced stability of CARP proteins and affected contractility parameters of cardiac muscles in the engineered heart tissues,[81] demonstrating that functional impacts of *ANKRD1* mutations in cardiac hypertrophy might be quantitatively and qualitatively different *in vivo*.

On the other hand, there are many other disease genes for DCM, which may be categorized into several groups. The first group includes mutations in genes for nuclear membrane-associated proteins, lamin A/C (*LMNA*)[82,83] and emerin (*EMD*),[84] which cause autosomal dominant and X-linked Emery-Dreifuss muscular dystrophy,[85] respectively. Although molecular mechanisms underlying the development of DCM caused by the nuclear membrane abnormality remain not

fully understood,[86] a study of a *LMNA* mutation knock-in mouse[87] showed that the mutation activated the MAPK pathway, suggesting an impaired signal transduction was involved in the pathogenesis of DCM.[88] The second group includes mutations affecting ion channel function; regulatory subunit of ATP-sensitive potassium channel (*ABCC9*)[89] and cardiac sodium channel (*SCN5A*).[90] Clinical phenotypes of *ABCC9* and *SCN5A* mutations were DCM accompanied by ventricular tachyrcardia[89] and conduction defects,[90] respectively. It should be noted here that the channelopathy is etiologically overlapping with the cardiomyopathy, such as *SCN5A* mutations in DCM and long QT syndrome, *CAV3* mutations in HCM and long QT syndrome, and *RYR2* mutations in ARVC and catecholaminergic polymorphic ventricular tachycardia.[91] The third group is composed of mutations in genes for titin-N2B interacting proteins, four and half LIM protein (*FHL2*)[92] and αB-crystallin (*CRYAB*).[77] Because a titin-N2B region mutation found in DCM reduced binding to FHL2[76] and a *FHL2* mutation reduced binding to titin-N2B,[92] impaired interaction between titin and FHL2 was associated with DCM. Molecular mechanisms underlying this phenomenon may be that FHL2 function as a tethering molecule of adenylkinase, phosphofructokinase and muscle creatine kinase, that is, proper recruitment of metabolic enzymes was impaired, although abnormality in other functions of FHL2[93] could not be neglected. The DCM-associated *CRYAB* mutation decreased binding to titin-N2B region and a DCM-associated titin-N2B region mutation decreased binding to αB-crystallin,[77] suggesting that impaired interaction between titin-N2B and αB-crystallin resulted in DCM. However, another HCM-associated titin-N2B mutation also reduced the binding to αB-crystallin.[76] It is not clarified why the impaired binding of titin-N2B and αB-crystallin could exert both HCM and DCM phenotypes. There might be additional factors involved in the phenotypic expression of titin-N2B mutations, such that binding to FHL2 was different between the HCM- and DCM-associated mutations and that the DCM-associated titin-N2B mutation was a truncation mutation, whereas the HCM-associated mutation was a missense mutation.[76] The fourth group is related to intracellular Ca^{2+} handling. Phospholamban is an inhibitory molecule of SERCA, which is activated when phosphorylated by protein kinase A.[94] Functional analysis of phospholamban gene (*PLN*) mutations found in DCM showed that the mutations rendered phospholamban constitutive active, that is, inhibiting SERCA.[95,96] In contrast, a truncation mutation of *PLN*, that is, loss of *PLN* function, is reported in familial HCM.[97] Although *PLN* deficiency in mice resulted in enhanced contractility,[98] no cardiac hypertrophy was observed in the mice. In addition, loss of *PLN* rescued DCM phenotype[99] in *CSRP3* knockout mice, and a dominant-negative form of *PLN* prevented heart failure in cardiomyopathic hamster BIO14.6,[100] which is known to be caused by *SAGD* deficiency.[101] These observations suggest that functional impairment of phospholamban may prevent systolic dysfunction but not directly involved in the cardiac hypertrophy. Moreover, promoter mutations of *PLN*, which increased transcription, were recently reported in HCM.[102,103] Furthermore, we recently reported a *FHOD3* mutation in DCM.[104] *FHOD3* is preferentially expressed in the heart and has a role in cellular actin polymerization in myofibrinogenesis and may be involved in repair of disrupted actin fibers. In cytoplasm, actin molecules can be found in both monomer and polymer forms, of which cellular stress induces actin polymerization; the process is called as actin dynamics. Monomeric actin is known to associate with a SRF coactivator MAL/MRTF-A/MKL1, and when actin polymerization would occur MAL/MRTF-A/MKL1 binds SRF to translocate into nucleus leading to induction of SRF-dependent gene expression.[104] Functional assay showed that the *FHOD3* mutation found in DCM impaired the actin dynamics-dependent SRF activation,[104] suggesting that maladaptive cardiac remodeling would occur in the presence of *FHOD3* mutation. In addition, mutations in Bcl2-associated athanogene 3 (*BAG3*) were reported as the cause of DCM.[105,106] Our functional analyses demonstrated that DCM-associated *BAG3* mutations render cardiomyocytes susceptible to metabolic stress leading to apoptosis, which was not observed for another myofibrillar myopathy-associated *BAG3* mutaion.[106]

There are further other mutations reported in DCM patients—G4.5 gene (tafazzin, *TAZ*, Barth's syndrome),[107] fukutin gene (*FKTN*),[108] desmoplakin gene (*DSP*)[109] and plakoglobin gene (*JUP*)[110] mutations. These mutations, however, were found in 'syndromic' DCM that is accompanied by disorders and/or dysfunction in skeletal muscle, skin or hair. An example is that *FKTN* mutation was not found in pure DCM, but was found in skeletal myopathy accompanied by DCM and an early sign of *FKTN* mutation-associated DCM was hyper-CKemia.[111]

GENETIC MODIFIERS FOR CARDIOMYOPATHY

It is well known that carriers of gene mutations do not always develop disease even in the monogenic disease and this phenomenon is called as 'penetrance' of disease-causing mutation. Penetrance is defined by the frequency of subjects with disease manifestation among mutation carriers. It is well known for HCM that the penetrance could vary with age, gender, physiological performance or exercise depending on each mutation. In addition, both intra- and inter-familial phenotypic variation can be observed for each mutation and this variation is explained at least in part by the genetic background, genetic modifiers, which modify the expressivity of disease-causing mutations.[112–114] Because the identification of such modifier genes is important and useful in predicting clinical course of each patient with specific mutation, various attempts have been performed to find out gene polymorphisms associated with disease progression since the early era soon after the identification of disease-causing mutations in HCM. Although there are several initial reports noting, for example, the association of gene polymorphisms in RAS cascade,[112,113,115] such studies were not designed for comparison of polymorphisms in subjects between the population with and without specific mutation and hence it was difficult to assess the modifying effect of each polymorphism in modifying disease expression of a specific mutation. In this regard, it is needed to investigate the phenotypic expression as a quantitative trait to identify disease-modifying loci in the population with specific mutation. However, such a study will require a large cohort of patients with cardiomyopathy carrying the specific mutations and it is almost impossible to design a human study. Therefore, animal models carrying a specific mutation would be required and there are several reports addressing the issue of such attempts. It has been reported as early as in 2001 that cardiac hypertrophy in mice carrying the *MYH7* Arg403Asn mutation were considerably different in mouse strains, although responsible loci was not identified.[116] It has recently been reported from the analysis of transcriptome in mice with the *MYH7* Arg403Asn mutation that a four-and-a-half LIM domain protein 1 gene (*FHL1*) has a key role in modifying the cardiac phenotype.[117] It has also been speculated that *FHL1* might be a genetic factor to determine the gender difference in the cardiac phenotype of HCM observed in this model mice.[117] In addition, systematic search for genetic loci involved in the strain-specific cardiac phenotypes in calsequestrin gene (*CSQ*) overexpressing transgenic mice showing DCM phenotype has identified at least seven different quantitative trait

loci,[118–120] among which only one locus was clarified as cardiac troponin I-interacting kinase gene (*TNNI3K*) at the gene level.[121]

Another issue of modifier is the gender difference. It is well known that males are more susceptible to cardiomyopathy than females, because male to female ratios in patients with HCM and DCM are 2.3 and 2.6, respectively, and male patients suffer from more severe cardiac hypertrophy and faster disease progression in HCM[122] and heart failure in DCM, especially in DCM caused by *LMNA* mutations,[123] than female patients. Molecular basis of the gender difference in HCM has not been deciphered, but it was speculated in the mouse model with the *MYH7* Arg403Asn mutation that *FHL1* might have a role in the gender difference, because it is located on the X chromosome.[119] As for the gender difference in the *LMNA*-linked DCM, we recently revealed that the male sex hormone was responsible for worse prognosis of DCM from the analysis of a mouse model, in which a *LMNA* mutation, H222P, was knocked-in. The model mice developed DCM with gender difference[87] and it was revealed that androgen receptor (AR) was translocated into nuclei in the absence of testosterone, when cardiomyocytes carried the *LMNA* H222P mutation.[124] In addition, the nuclear translocation of AR could be observed with another *LMNA* mutation, R225X, which was identified in a DCM family in which male patients developed DCM earlier than female patients. Because the nuclear translocation of AR was depending on FHL2 and associated with SRF, resulting in the activation of SRF-dependent transcription of cardiac remodeling associated genes, it was suggested that the gender difference in the *LMNA*-linked DCM was a consequence of abnormal regulation of remodeling genes in the presence of androgens.[124] It should be noted here that an AR blocker, flutamide, could ameliorate DCM in the mouse model, anti-androgen therapy might prevent progression of heart failure in specific cases with *LMNA*-linked DCM, although the nuclear translocation of AR was not observed with another *LMNA* mutation, delK32, which did not show apparent gender difference in a knock-in model mice.[124,125]

MUTATIONS IN OTHER CARDIOMYOPATHIES

Disease-causing gene mutations can also be identified in other cardiomyopathies. For example, mutations in sarcomere proteins were found in RCM (Table 1). It is interesting to note that *MYH7*, *TNNT2* and *TNNI3* mutations were associated with RCM, HCM and DCM. Molecular basis of the differences between RCM-associated mutations and HCM-associated mutations was that the RCM-associated mutations showed much greater Ca^{2+} sensitization than the HCM-associated mutations, as demonstrated for *TNNT2*[126] and *TNNI3*[127] mutations. In accordance with these findings, it was reported that restrictive phenotype (RCM-like HCM) was uncommon in HCM and may represent a poor prognosis form with severe diastolic dysfunction.[128] On the other hand, the mechanistic difference between RCM-associated mutations and DCM-associated mutations is not elucidated, but a gene dose effect could be involved in the difference, because RCM-associated *TNNI3* mutation was found in heterozygous state,[129] whereas the DCM-associated *TNNI3* mutation was found in homozygous state.[130]

LVNC is a form of cardiomyopathy in which ventricular trabeculations was poorly developed, and mutations in *MYH7*,[131] *CACT*,[132] *DES*,[53] *LMNA*,[133] *TAZ*,[134] *DTNA*[135] and *LDB3*[51] were reported in LVNC (Table 1). Molecular mechanisms of the mutations in causing LVNC are not elucidated. In a mouse model, deficiency of BMP10 resulted in the LVNC phenotype.[136] BMP10 is a member of the TGFβ family, which is expressed mainly in the heart, and has a key role in morphogenesis of the heart.[137] Therefore, LVNC might be a developmental error in the hearts carrying the mutations in components of sarcomere and/or sarcolemma.

Another primary cardiomyopathy AVRC has also been investigated for mutations[138] (Table 1). Because the ARVC-associated mutations can be found in genes for plakoglobin (*JUP*),[139] desmoplakin (*DSP*),[140] plakophilin-2 (*PKP2*)[141] and desmoglein (*DSG3*),[142] they were considered to disrupt cell–cell contacts via desmosomes. *RYR2* mutations were also reported in ARVC,[143] linking cardiomyopathy to channelopathy. Promoter variant of TGFβ3 was also reported in ARVC,[144] but its pathological significance remains to be resolved.

CONCLUSION REMARKS

In this review, gene mutations found in the hereditary cardiomyopathy are summarized. Each family or patient has usually only one disease-causing mutation, but the primary cardiomyopathy is both clinically and etiologically heterogeneous even in a specific clinical type. Because different causes result in the same phenotype, there might be several pathways in the pathogenesis of primary cardiomyopathy, such that abnormalities in the Ca^{2+} sensitivity, stretch response and metabolic stress response. Intervention of these common pathways will be a therapeutic or preventive strategy for hereditary cardiomyopathy caused by different mutations.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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