Circ J 2008; **Suppl A:** A-38 – A-48

Molecular Etiology and Pathogenesis of Hereditary Cardiomyopathy

Akinori Kimura, MD

Cardiomyopathy is defined as a cardiac disease caused by functional abnormality of cardiac muscle, and the etiology of the functional abnormality includes both extrinsic and intrinsic factors. Cardiomyopathy caused by the intrinsic factors is defined as idiopathic or primary cardiomyopathy, and there are several clinical phenotypes, including hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM). The major intrinsic factor is gene mutations, and linkage studies, as well as candidate gene approaches, have deciphered multiple disease genes for hereditary primary cardiomyopathy. Of note is that mutations in the same disease gene can be found in different clinical phenotypes of cardiomyopathy. Functional analyses of disease-related mutations have revealed that characteristic functional alterations are associated with the clinical phenotypes, such that increased and decreased Ca^{2+} sensitivity because of sarcomere mutations are associated with HCM and DCM, respectively. In addition, recent data have suggested that mutations in the Z-disc components found in HCM and DCM may result in increased and decreased stiffness of the sarcomere (ie, stiff sarcomere and loose sarcomere, respectively). More recently, mutations in the components of the I region can be found in hereditary cardiomyopathy, further complicating the etiology of primary cardiomyopathy. (*Circ J* 2008; **Suppl A:** A-38 – A-48)

Key Words: Calcium sensitivity; Cardiomyopathy; Sarcomere; Stretch response; Z-disc

Cardiomyopathy is a heterogeneous disease caused by functional abnormality of cardiac muscle and is classified into primary cardiomyopathy and secondary cardiomyopathy[1] Secondary cardiomyopathy is defined as cardiomyopathy caused by extrinsic factors including ischemia, hypertension and metabolic disorders, whereas the diagnosis of primary cardiomyopathy is based on the exclusion of secondary cardiomyopathy and there are several different clinical subtypes[2,3] Hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) are the 2 major clinical phenotypes of primary cardiomyopathy that have been defined as "idiopathic" (ie, of unknown etiology). HCM is characterized by left ventricular (LV) hypertrophy, often asymmetric, accompanied by myofibrillar disarray and reduced compliance (diastolic dysfunction) of cardiac ventricles. Cardiac hypertrophy in HCM, as in the case of other cardiac diseases accompanied by cardiac hypertrophy, is thought to be a consequence of compensation for functional deficits in demand of overload. HCM is a major cause of sudden death in the young and of heart failure. In contrast, DCM is characterized by a dilated ventricular cavity with systolic dysfunction. Clinical symptoms of DCM are those of heart failure and it is often associated with sudden death. There are other clinical phenotypes of cardiomyopathy. Restrictive cardiomyopathy (RCM) is accompanied by increased stiffness of the myocardium with diastolic dysfunction without significant hypertrophy[3] In addition, arrhythmogenic right ventricular cardiomyopathy (ARVC) is characterized by a dilated, dysfunctional right ventricle (RV), ventricular arrhythmias, and fibrofatty replacement of the RV. Another cardiomyopathy has been recently classified: LV noncompaction (LVNC) has trabeculations in the LV, as well as hypertrophy and/or dilation[1]

The etiology of primary cardiomyopathy has been unknown, but in the past 2 decades, considerable effort has been made to identity the genetic abnormality associated with HCM and/or DCM. Familial occurrence has been noted in RCM, ARVC and LVNC[1–4] and it is known that more than 50% of HCM patients have a family history of the disease, consistent with an autosomal dominant genetic trait[5] As well, approximately 20–35% of DCM patients have a family history of the disease, mainly consistent with autosomal dominant inheritance, although some familial cases may be explained by autosomal recessive or X-linked recessive trait, indicating that DCM is more genetically heterogeneous than HCM[6,7] Linkage studies have been performed in multiplex families with primary cardiomyopathy to identify the disease loci in each family, which enables deciphering of the disease-related or disease-linked mutations in the genes located within the loci. Subsequently, other candidate gene analyses, focusing on the genes encoding for proteins related to or interacting with the products of the disease genes for primary cardiomyopathy, have been successful in unraveling novel disease genes. As shown in Table 1 many disease genes have been identified. The most important point is the overlapping of disease genes for the different clinical phenotypes of primary cardiomyopathy.

In this review, identification of disease gene mutations for hereditary cardiomyopathy and their functional abnormalities in relation to pathogenesis will be discussed.

(Received January 16, 2008; accepted June 22, 2008; released online September 4, 2008)
Department of Molecular Pathogenesis, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan
Mailing address: Akinori Kimura, MD, Department of Molecular Pathogenesis, Medical Research Institute, Tokyo Medical and Dental University, Tokyo 113-8510, Japan. E-mail: akitis@mri.tmd.ac.jp

Table 1 Disease Genes for Hereditary Cardiomyopathy

Clinical phenotype	*Mode of inheritance*	*Gene*	*Coding protein*
HCM/DCM/RCM/LVNC	*AD*	*MYH7*	*Cardiac β-myosin heavy chain*
HCM/DCM/RCM	*AD*	*TNNT2*	*Cardiac troponin T*
HCM/DCM	*AD*	*TPM1*	*α-tropomyosin*
HCM/DCM	*AD*	*MYBPC3*	*Cardiac myosin binding protein-C*
HCM	*AD*	*MYL3*	*Ventricular myosin essential light chain*
HCM	*AD*	*MYL2*	*Ventricular myosin regulatory light chain*
HCM/DCM/RCM	*AD*	*TNNI3*	*Cardiac troponin I*
HCM/DCM/LVNC	*AD*	*ACTC*	*Cardiac α-actin*
HCM/DCM	*AD*	*TTN*	*Titin, connectin*
HCM/DCM	*AD*	*TNNC1*	*Cardiac troponin C*
HCM	*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*	*JPH-2*	*Junctophilin-2*
HCM	*AD*	*OBSCN*	*Obscurin*
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*	*PLB*	*Phospholamban*
DCM	*AD*	*ABCC9*	*KATP channel*
DCM	*AD*	*SCN5A*	*Cardiac Na channel*
DCM/HCM	*AD*	*CRYAB*	*αB crystallin*
DCM	*AD*	*FHL2*	*Four-and-a-half LIM protein-2, FHL2*
DCM	*AD*	*LMNA4*	*Laminin α4*
DCM	*AD*	*MYPN*	*Myopalladin*
DCM	*XR*	*DMD*	*Dystrophin*
DCM	*XR*	*EMD*	*Emerin*
LVNC/DCM	*XR*	*TAZ*	*Tafazzin, G4.5*
DCM	*XR*	*FKTN*	*Fuktin*
ARVC/DCM	*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*
LVNC	*AD*	*DTNA*	*α-dystrobrebin*
HCM-like	*AD*	*PRKAG2*	*γ2 subunit of AMP activated protein kinase*
Fabry disease	*XR*	*GALA*	*α-galactosidase*
Pompe disease	*AR*	*GAA*	*α1,4-glucosidase*
Danon disease	*XR*	*LAMP2*	*Lysosome-associated membrane protein 2*

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

HCM

Sarcomere Mutations in HCM

The first demonstration of a disease-causing gene was the identification of a missense mutation in the cardiac β-myosin heavy chain gene (*MYH7*), which was linked to HCM in a large multiplex family[8] Subsequently, many investigators, including us, have investigated *MYH7* for mutation in patients with HCM and many different missense mutations have been identified as the cause of HCM. However, the frequency of *MYH7* mutations in HCM patients is at most approximately 30% and there are many families in which HCM was not linked to the *MYH7* locus. Linkage studies in non-*MYH7*-linked HCM families have revealed that mutations in the α-tropomyosin gene (*TPM1*), cardiac troponin T gene (*TNNT2*) and cardiac myosin binding protein-C gene (*MYBP3*). Because these genes encode for components of the sarcomere involved in muscle contraction, genes for other components were analyzed for mutations in HCM patients, leading to the identification of mutations in the ventricular myosin essential light chain gene (*MYL3*), ventricular myosin regulatory light chain gene (*MYL2*), and cardiac troponin I gene (*TNNI3*) in different HCM patients. Mutations in genes for cardiac α-actin gene (*CACT*) and cardiac troponin C (*TNNC1*) were also reported as the cause of HCM. Therefore, mutations in any component of the sarcomere can result in HCM[3,5]

As shown in Table 2, sarcomere mutations can be found in approximately 40% of Japanese patients with familial HCM in the heterozygous state, consistent with autosomal dominant inheritance. Approximately 20%, 10%, and 10% of patients carried mutations in *MYH7*, *TNNT2*, and *MYBPC3*, respectively, while a few cases had mutations in other components of the sarcomere, such as *MYL2*, *MYL3*, and *TNNI3*. So far, we have not found any patients with mutations in 2 or more disease genes, though there have been some patients who are homozygous for the sarcomere mutation. The homozygous patients show a more severe clinical mani-

Table 2 Frequencies of Disease-Associated Mutations in Japanese and Korean Patients With HCM

Gene	Familial cases (%, n=162)	Sporadic cases (%, n=100)
MYH7	19.1	2.0
TNNT2	11.7	3.0
TPM1	0.6	0.0
MYBPC3	11.1	5.0
MYL3	0.6	1.0
MYL2	1.2	0.0
TNNI3	2.5	3.0
ACTC	0.0	0.0
TTN[#]	>1.9	>1.0
CSRP3	0.0	0.0
TNNC1	0.0	0.0
CAV3	0.6	0.0
MYH6	NT	NT
TCAP	1.2	0.0
CRYAB	0.0	0.0
VCL	0.0	0.0
JPH-2	NT	NT
OBSCN	0.6	0.0
Sum	>50.0	>15.0

[#]*Z-disc, N2-B, N2-A, Novex3 and is2 domains (≈20% of entire TTN) were analyzed.*
NT, not tested. Other abbreviation see in Table 1.

Table 3 Phenotypic Expression of Japanese HCM Patients Carrying Mutations in *MYH7*, *TNNT2* or *MYBPC3*

	MYH7 mutation	TNNT2 mutation	MYBPC3 mutation
No. of patients	41	30	41
No. of mutations	16	5	9
IVS (mm)	19.3±7.6	15.9±5.0	18.0±5.7
PW (mm)	11.1±2.6	10.3±5.0	11.0±3.0
LVDd (mm)	44.0±8.4	49.7±9.9	44.2±8.2
LAD (mm)	40.8±9.4	40.6±10.3	38.7±7.9
%FS	37.7±11.1	30.3±9.1	35.7±11.1
%EF	73.5±14.0	64.3±14.9	71.2±14.2
Age at diagnosis (years)	36.5±18.7	41.2±17.6	39.7±15.8
Duration of follow-up (years)	10.0±8.0	11.2±5.7	7.7±6.2
Prognosis (%)			
Improved	0.0	3.3	0.0
No change	59.5	25.0	64.1
Worse	24.3	42.9	28.2
Death	16.2	28.6	7.7
Worse + death	40.5	71.4	35.9
Death rate (%/year)	1.5	2.5	1.1

IVS, thickness of intraventricular septum; PW, thickness of posterior ventricular free wall; LVDd, diastolic left ventricular dimension; LAD, left atrial dimension; FS, fractional shortening; EF, ejection fraction.

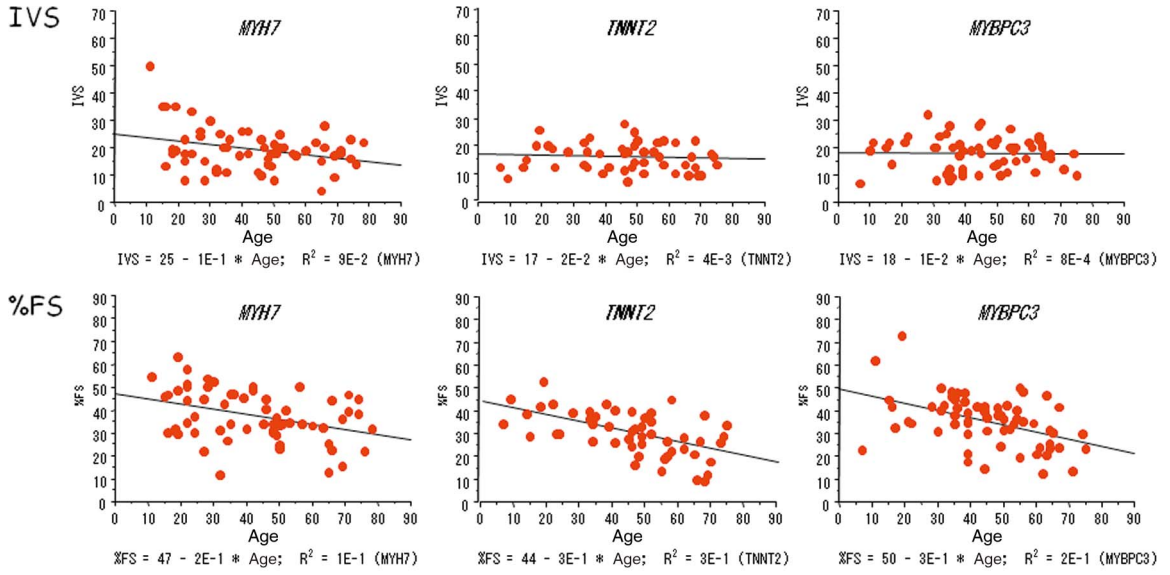

Fig 1. Clinical parameters of hypertrophic cardiomyopathy in patients carrying mutations in *MYH7*, *TNNT2*, or *MYBPC3*. (Upper) Correlation between age at examination and thickness of intraventricular septum (IVS) among patients with mutations in *MYH7* (Left), *TNNT2* (Middle) and *MYBPC3* (Right). (Lower) Correlation between age and % fractional shortening (%FS) among patients with the mutations.

festation than the heterozygous patients in a family, demonstrating the gene dose of mutation[9] Disease-related mutations can also be found in sporadic HCM. We found a de novo case;[10] but other sporadic cases are probably caused by the low penetrance of the mutation (ie, individuals carrying the disease-related mutations do not always develop the clinical manifestation of HCM, because most of the mutations found in patients with sporadic HCM can also found in other patients with familial HCM).

Genotype–phenotype correlation is the most important issue in the clinical implication of the identified disease genes. Clinical phenotypes of HCM caused by sarcomere mutations are in general different from each other, but there are some genotype–phenotype correlations![11] As a collaborator study of the Research Committee for Idiopathic Cardiomyopathy supported by the Ministry of Health, Labour and Welfare, Japan, we have investigated the clinical findings of Japanese patients who have been followed for relatively long periods and found to have sarcomere mutations. Although the phenotypes were considerably different among the different cases, several interesting tendencies were revealed (Table 3). Cardiac hypertrophy was more prominent in *MYH7* and *MYBPC3* cases than in *TNNT2* cases. It may be worth noting that the age at diagnosis was relatively late

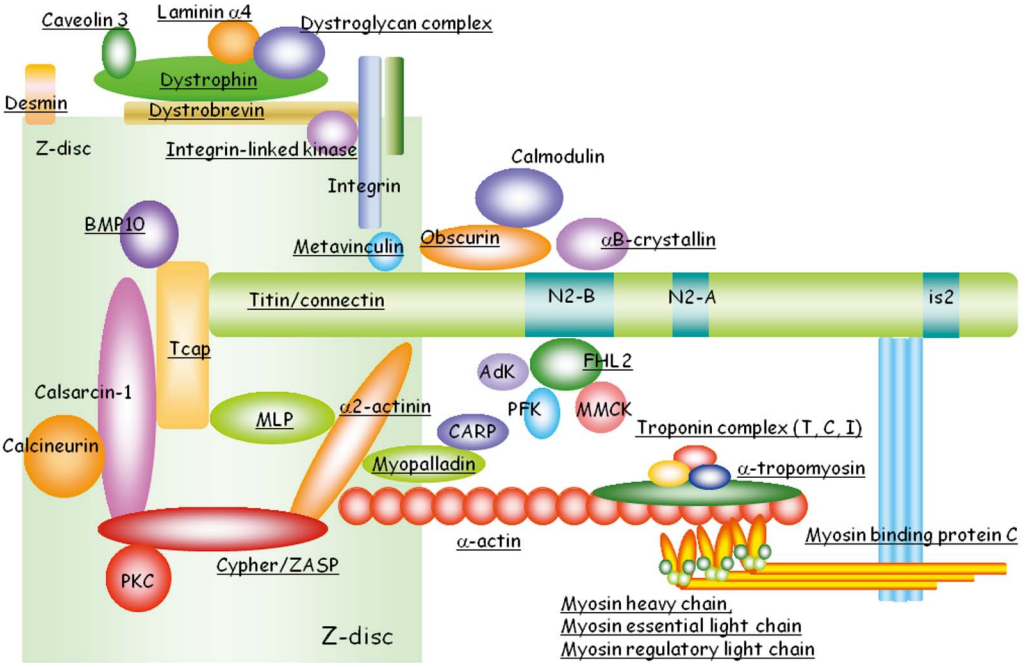

Fig 2. Schematic representation of the components of a half sarcomere. Components in which cardiomyopathy-associated mutations are found are underlined.

and cardiac function at diagnosis was relatively low in *TNNT2* cases than in the others. More importantly, the prognosis of *TNNT2* cases was worse than for the others; 43% and 29% of the *TNNT2* cases were dead or clinically worse, respectively, during the follow-up period (11.2±5.7 years), as compared with 24% and 16% of *MYH7* cases (10.0±8.0 years). Although *MYBPC3* cases were initially reported to follow a relatively benign clinical course[12] our data demonstrated that 36% of such cases had a worse prognosis during the follow-up period (7.7±6.2 years). In general, cardiac hypertrophy at diagnosis gradually reduced during the follow-up period, as well as cardiac function decreasing later in life, even in the *MYBPC3* cases (Fig 1). The annual death rate after the diagnosis was 2.5%, 1.5% and 1.1% for *TNNT2*, *MYH7*, and *MYBPC3* cases, respectively, demonstrating that *TNNT2* cases had the worst prognosis and that the long-range prognosis of *MYBPC3* cases was not at all benign![13]

Initial analyses of the functional changes caused by the *MYH7* mutations demonstrated decreased power generation by the mutant myosin heavy chains[14] and the identification of HCM-related mutations in sarcomere components lead to a hypothesis that HCM is a disease of the sarcomere and that cardiac hypertrophy was a compensation for decreased power generation![15] However, mutations in *TNNI3* were found at the contraction inhibitory domain![10] implying that decreased power might not be a common functional change caused by the sarcomere mutations. Indeed, subsequent functional analyses of mutations in sarcomere components other than the myosin heavy chain have revealed that contractile performance was not always decreased by the mutations![16] Instead, it was demonstrated that most HCM-associated sarcomere mutations resulted in increased Ca^{2+} sensitivity of muscle contraction![16–20] Because a myosin heavy chain mutation, which causes HCM in transgenic mice, also shows increased Ca^{2+} sensitivity at the muscle fiber level;[21] a common functional alteration caused by HCM-related mutations may be increased Ca^{2+} sensitivity. Muscle contraction depends on the concentration of intracellular Ca^{2+}, which is released from the sarcoplasmic reticulum (SR) via the ryanodine receptor (RyR2) and retaken up into the SR via the SR Ca^{2+}–ATPase (SERCA). When the concentration of Ca^{2+} is increased or decreased, muscle contracts or relaxes, respectively. Increased Ca^{2+} sensitivity is a leftward shift of the Ca^{2+}-tension curve; either more tension is generated by the mutant sarcomere than the normal sarcomere at the same Ca^{2+} concentration (hypercontraction) or muscle with mutant sarcomeres is less relaxed (diastolic dysfunction) than the normal sarcomere. This is consistent with the finding that the characteristic features of HCM are hypercontraction and diastolic dysfunction.

Z-Disc Mutations in HCM

Because sarcomere mutations have been found in less than half of familial HCM patients, there must be other disease gene(s) for HCM. We and others have taken candidate gene approaches to identify disease-related mutations in other genes expressed in cardiomyocytes and successfully identified various disease genes (Fig 2). Identification of a HCM-associated mutation in the titin gene (*TTN*)[22] was the first example of a disease gene other than the sarcomere components. Functional alteration by the *TTN* mutation caused increased binding to α-actinin[22] (Fig 3). In addition, HCM-associated Tcap gene (*TCAP*) mutations were found to increase binding to titin, MLP, and carsarcin[23] (Fig 4), leading to a hypothesis that Z-disc mutations in HCM may result in increased binding of Z-disc components and hence a "stiff sarcomere";[23] which would increase passive tension upon stretching of the sarcomere. Because the increased passive tension is associated with increased Ca^{2+} sensitivity;[24–26] we have speculated that HCM-associated abnormalities in both the Z-disc components and sarcomere components cause increased Ca^{2+} sensitivity. A possible controversy ex-

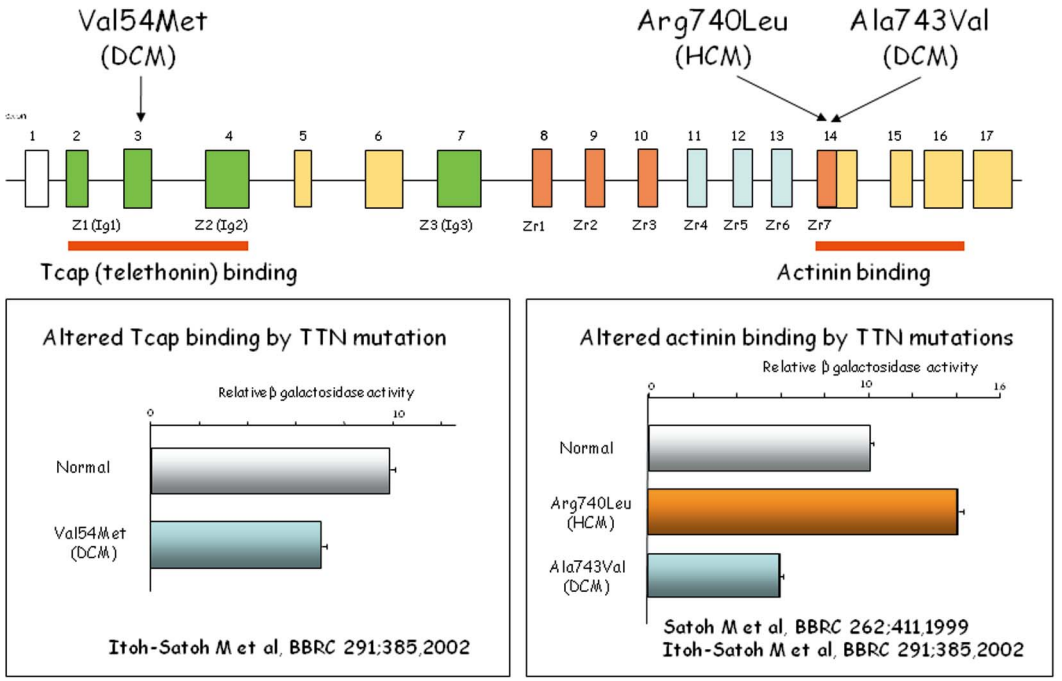

Fig 3. Functional alterations caused by *TTN* mutations found in hypertrophic cardiomyopathy (HCM) or dilated cardiomyopathy (DCM) patients. (Upper) *TTN* mutations in the Z-disc domain are shown with the exon–intron structure. Exons encoding for binding domain to Tcap and actinin are shown. (Lower) Semi-quantitative analysis of the binding of titin with Tcap or actinin in the presence or absence of mutations, as assessed by yeast-2-hybrid or mammalian-2-hybrid analysis expressed by β-galactosidase activity.[22,56]

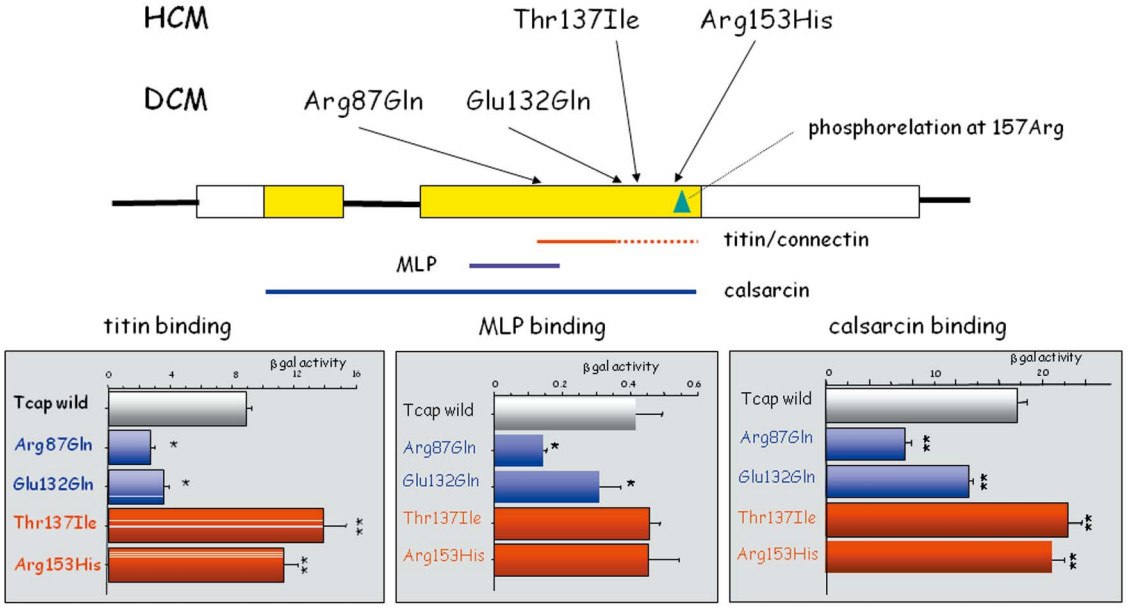

Fig 4. Functional alterations caused by *TCAP* mutations found in hypertrophic cardiomyopathy (HCM) or dilated cardiomyopathy (DCM) patients. (Upper) *TCAP* mutations are shown with the exon–intron structure. Domain structures are represented. (Lower) Semi-quantitative analysis of binding of Tcap with titin (Left), MLP (Middle) and calsarcin (Right) in the presence or absence of mutations, as assessed by mammalian-2-hybrid analysis expressed by β-galactosidase activity.[23] *p<0.01, **p<0.001. Hayashi T et al, JACC 44: 2192, 2004.

ists; that is, HCM-associated MLP gene (*CSRP3*) mutations are reported to decrease binding to α-actinin and N-RAP;[27,28] however as discussed later, DCM-associated mutations have been found in *CSRP3* and the α-actinin gene (*ACTN2*) and these mutations decreased binding to each other.[29] Therefore, decreased binding between MLP and α-actinin is associated with both HCM and DCM. This discrepancy needs to be resolved by further studies.

Other Mutations in HCM

Several other disease genes for HCM have been reported, including mutations in the αB-crystalline gene (*CRYAB*);[30]

caveolin-3 gene (*CAV3*);[31] meta-vinculin gene (*VCL*);[32] junctophilin-2 gene (*JPH-2*);[33] and most recently the obscurin gene (*OBSCN*).[34] Functional analyses have been reported for *CRYAB*;[30] *CAV3*[31] and *OBSCN*[34] mutations, in which *CRYAB* aggregates αB-crystalline in the cytoplasm, *CAV3* decreases the cell surface expression of caveolin-3, and *OBSCN* decreases binding to titin. It is unclear how the aggregated αB-crystalline results in cardiac hypertrophy, but an impaired stress response may exaggerate the hypertrophic response.[30,35] It should be noted that an HCM-associated *TTN* mutation in the N2B region increased binding to FHL2[36] and decreased binding to αB-crystalline.[37] It has been reported that cell surface expression of caveolin-3 is associated with cardiac hypertrophy;[38] and also reported that overexpression of caveolin-3 inhibits the hypertrophic response;[39] suggesting that reduced caveolin-3-mediated signaling would result in cardiac hypertrophy. The function of obscurin is not fully understood, but it may be involved in calmodulin/CaMK-mediated signaling because obscurin was reported to bind and tether calmodulin to titin,[40] a process that was impaired by the HCM-associated *OBSCN* mutation.[34]

DCM

Membranous and Cytoskeletal Mutations in DCM

The first discovered disease gene for DCM was a mutation in the dystrophin gene (*DMD*) found in male siblings of X-linked DCM.[41] X-linked DCM is a rare form of familial DCM that almost exclusively affects males.[42] *DMD* mutations are well known to cause Duchenne type and Becker type muscular dystrophy. In general, muscular dystrophy affects skeletal muscles and cardiac involvement is usually observed later in the clinical course.[43,44] However, X-linked DCM cases usually manifest with cardiac symptoms and subtle skeletal muscle involvement.[42] Phenotypic variance in the *DMD* mutation may be caused by the domain of dystrophin that is affected.[42–44] As shown in Table 4, *DMD* mutations were found in 5% of sporadic DCM cases in our data. None of these patients showed skeletal muscle symptoms, demonstrating that X-linked DCM should be considered not only for male-sibling familial DCM cases, but also male sporadic DCM.

Dystrophin is a characteristic protein of muscles and plays a role in the mechanical linkage of the extracellular matrix to the intracellular cytoskeleton, in association with other proteins, forming the dystroglycan complex (DGC).[45] Because muscle contraction deforms and shortens the myocytes, the myofilaments need to be tightly connected to the membrane and extracellular matrix via DGC to properly transmit the generated force and to avoid damaging the cell membrane. Components of DGC in skeletal and cardiac muscles include dystrophin, dystroglycans (α and β), laminin αs, sarcoglycans (α, β, γ, and δ), dystrobrebins (α and β), syntrophin, and caveolin-3. In addition to DGC, integrins (α and β) are concentrated at the costemeres that overly the Z-lines in striated muscle, and the integrin complex also plays a role in the mechanical links for power transmission.[45] Therefore, abnormalities in the DGC and integrin complexes may result in muscular dystrophy and cardiomyopathy. Indeed, mutations in the δ-sarcoglycan gene (*SAGD*);[46] laminin α4 gene (*LMNA4*)[47] and integrin-linked kinase gene (*ILK*)[47] were found to cause DCM of autosomal dominant inheritance (Table 1). In addition, a *CAV3* mutation was found in HCM, as described above, and mutations in α-dystrobrebin were found in LVNC.[48] It was previously proposed that DCM was a disease of the cytoskeleton or its interacting proteins;[49] but recent studies have demonstrated that etiology of DCM is not confined to abnormalities of the cytoskeleton-related proteins, as described in the following sections.

Table 4 Frequencies of Disease-Associated Mutations in Japanese and Korean Adult Patients With DCM

Gene	*Familial cases (%, n=48)*	*Sporadic cases (%, n=100)*
ACTC	*0.0*	*0.0*
DES	*2.1*	*0.0*
DMD	*0.0*	*5.0*
LMNA	*0.0*	*NT*
SAGD	*0.0*	*NT*
MYH7	*0.0*	*0.0*
TNNT2	*0.0*	*0.0*
TPM1	*0.0*	*0.0*
TTN[#]	*>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*
LMNA4	*0.0*	*0.0*
Sum	*>16.7*	*>7.0*

[#]Z-disc, N2-B, N2-A, Novex3 and is2 domains (≈20% of entire TTN) were analyzed.
Abbreviations see in Tables 1,2.

Sarcomere Mutations in DCM

Identification of *CACT* mutations was the first discovery of a genetic cause of autosomal dominant DCM.[50] Following that report, a *CACT* mutation was also found in HCM;[51] demonstrating that sarcomere mutations cause both HCM and DCM (ie, overlap of disease genes for primary cardiomyopathy). The molecular basis of different phenotypes caused by *CACT* mutations was suggested by the finding of DCM-associated mutations at the α-actinin interacting domain of actin;[50] while HCM-associated mutations were found at the interacting domain of myosin heavy chain.[51] On the other hand, recent data suggest that the folding property differs between the DCM-associated mutant actin and HCM-associated mutant actin.[52] Another example of overlapping disease genes was the identification of the *TNNT2* mutation in DCM.[53] Functional study of *TNNT2* mutations demonstrated the difference between the DCM- and HCM-associated mutations (ie, DCM-associated *TNNT2* mutation decreased Ca^{2+} sensitivity of muscle contraction, which is in clear contrast to the increased sensitivity caused by the HCM-associated mutation).[54] Therefore, sarcomere mutations can be found in both HCM and DCM, but differences in the functional alterations may determine the clinical phenotypes.[55]

Z-Disc Mutations in DCM

As shown in Table 4, mutations of membranous, cytoskeletal, or sarcomeric proteins were not found in our panel of familial DCM. Instead, mutations in Z-disc components were relatively frequent in Japanese familial DCM (Table 4). We have reported several DCM-associated Z-disc protein

gene mutations in *TTN*,[56] *CSRP3*,[57] *TCAP*[23,57] and the Cypher/ZASP gene (*LDB3*),[58] although the *CSRP3* mutation was not found in Japanese patients.[57] As described in the HCM section, *TCAP* mutations found in DCM show opposite functional alterations to the HCM-associated mutations.[23] Similarly, a DCM-associated *TTN* mutation found in the actinin-binding domain showed decreased binding to actinin[56] (Fig 3a). In addition, another DCM-associated *TTN* mutation found in the Tcap binding domain decreased binding to Tcap[56] (Fig 3a). Because the Z-disc element mutations result in decreased binding among the elements, we hypothesize that DCM is a disease of "loose sarcomeres". The loose sarcomere is evident in an animal model of DCM using *CSRP3* (MLP) knock-out mice, in which the Z-disc is wide and the stretch response is impaired.[57] Because the stretch response is a hypertrophic response of cardiomyocytes against passive tension, and Z-disc elements are suggested to be a stretch sensor of cardiomyocytes, abnormality in the Z-disc elements may alter the regulation of the stretch response.

Cypher/ZASP is a Z-disc element connecting calsarcin and actinin[58] (Fig 1). It is interesting to note that calsarcin binds calcineurin,[59] a well-known Ser/Thr phosphatase involved in the process of hypertrophy of cardiomyocytes.[60,61] The significance of calcineurin anchorage to the Z-disc is not fully understood, but it is involved in stress-induced calcineurin-NFAT activation, because heterozygous MLP knock-out mice show a reduction in NFAT activation, as well as dislocation of calcineurin from the Z-disc.[62] Cypher/ZASP binds protein kinase C (PKC),[63] and a DCM-associated *LDB3* mutation in the PKC binding domain was found to increase that binding,[58] suggesting that phosphorylation/dephosphorylation of Z-disc elements might be involved in the stretch response and pathogenesis of primary cardiomyopathy. Identification of target protein(s) for phosphorylation (by PKC)/dephosphorylation (by calcineurin) will unravel the molecular mechanism(s) of the stretch response and/or signaling molecule(s) of cardiac hypertrophy. Functional alterations found for mutations in Z-disc elements are schematically shown in Fig 4.

Several other *LDB3* mutations in DCM or LVNC have been reported.[64] Although the functional changes caused by these mutations remain unknown, it appears that the binding to PKC is not changed because they were not in the PKC interacting domain. In addition, DCM-associated mutations were found in genes for other Z-line associated proteins: desmin (*DES*)[65] and metavinculin (*VCL*).[66] The *VCL* mutation was showed to impair binding to actin,[66] whereas the *DES* mutations resulted in subtle changes in the cytoplasmic desmin network.[67] In addition, mutations in the myopalladin gene (*MYPN*) have recently been reported in DCM. Although the molecular mechanisms of *MYPN* mutations leading to DCM remain unclear, the mutations impair myofibrinogenesis.[68]

Other Mutations in DCM

The etiology of familial DCM is quite heterogeneous. In addition to those described above, there are several other disease genes for DCM categorized into groups. The first group includes mutations in genes for nuclear membrane proteins, lamin A/C (*LMNA*)[69,70] and emerin (*EMD*),[71] which cause autosomal dominant and X-linked Emery-Dreifuss muscular dystrophy (EDMD), respectively. EDMD manifests as muscular dystrophy and DCM associated with conduction block.[72] The molecular mechanisms underlying the development of DCM caused by nuclear membrane abnormality are not fully understood.[73]

The second group consists of mutations affecting ion channel function: the regulatory subunit of the ATP-sensitive potassium channel (*ABCC9*)[74] and the cardiac sodium channel (*SCN5A*).[75] Clinical phenotypes of *ABCC9* and *SCN5A* mutations are of DCM associated with ventricular tachyrcardia[74] and conduction defects,[75] respectively. It should be noted that channelopathy etiologically overlaps with cardiomyopathy, such as the *SCN5A* mutations in DCM and long-QT syndrome, the *CAV3* mutations in HCM and long-QT syndrome, and ryanodine receptor gene (*RYR2*) mutations in ARVC and catecholaminergic polymorphic ventricular tachycardia.[76]

The third group is composed of mutations in genes for titin-N2B interacting proteins, four and half LIM protein (*FHL2*)[77] and αB-crystallin (*CRYAB*).[37] Because a titin-N2B region mutation found in DCM reduced binding to FHL2, and the FHL2 mutation reduced binding to titin N2B, impaired interaction between titin and FHL2 appeared to result in DCM. The molecular mechanisms underlying this phenomenon may be that FHL2 functions as a tethering molecule of adenyl kinase, phosphofructokinase, and muscle creatine kinase (ie, proper recruitment of metabolic enzymes is impaired), although abnormality in other functions of FHL2[78] should not be neglected. The DCM-associated *CRYAB* mutation decreased binding to the titin N2B region and a DCM-associated titin N2B region mutation decreased binding to αB-crystallin,[37] suggesting that impaired interaction between titin N2B and αB-crystallin results in DCM. However, an HCM-associated titin N2B mutation also reduced the binding to αB-crystallin, and it is not clear why impaired binding of titin and αB-crystallin would express as both HCM and DCM. There may be additional factors involved in the phenotypic expression of titin N2B mutations, such that binding to FHL2 differs between the HCM- and DCM-associated mutations and that the DCM-associated titin N2B mutation is a truncation mutation, whereas the HCM-associated mutation is a missense mutation.[36]

The fourth group is related to intracellular Ca^{2+} handling. As discussed previously, muscle contraction correlates with Ca^{2+} concentration, and SERCA plays a key role in the re-uptake of intracellular Ca^{2+} to the SR, leading to relaxation of muscle. Phospholamban is an inhibitory molecule of SERCA, which is physiologically active when phosphorylated by protein kinase A.[79] Functional analysis of the phospholamban gene (*PLN*) mutations found in DCM shows that the mutation is constitutive active (ie, inhibiting SERCA).[80,81] In contrast, a truncation mutation of *PLN* (ie, loss of *PLN* function) was recently reported in familial HCM.[82] Although *PLN* deficiency in mice was reported to result in enhanced contractility,[83] cardiac hypertrophy did not occur in the mice. In addition, loss of *PLN* rescued the DCM phenotype[84] in MLP knockout mice and a dominant-negative form of *PLN* prevented heart failure in the cardiomyopathic hamster BIO14.6,[85] which is known to be caused by *SAGD* deficiency.[86] These observations suggest that functional impairment of PLN may prevent systolic dysfunction, but is not directly involved in cardiac hypertrophy. Moreover, promoter mutations of *PLN*, which increased transcription, were recently reported in HCM.[87,88] Because transgenic mice overexpressing *PLN* did not show cardiac hypertrophy, rather they showed systolic dysfunction,[89] the pathological significance of *PLN* promoter mutations in

human HCM remains to be clarified. The other mutations found in DCM include the G4.5 gene (tafazzin, *TAZ*, Barth's syndrome);[90] fukutin gene;[91] desmoplakin gene (*DSP*);[92] and plakoglobin gene (*JUP*).[93] These mutations, however, were not found in "pure" DCM, only in "syndromic" DCM accompanied by disorders and/or dysfunction in skeletal muscle, skin or hair.

Mutations in Other Cardiomyopathies

Disease-causing gene mutations can also be identified for other cardiomyopathies. As shown in Table 1, mutations in sarcomeric proteins have been found in RCM patients. It is interesting to note that *MYH7*, *TNNT2*, and *TNNI3* mutations are associated with RCM, HCM and DCM, respectively. The molecular basis of the differences between RCM-associated mutations and HCM-associated mutations is that the RCM-associated mutations show much greater Ca^{2+} sensitization, as demonstrated for the *TNNT2*[94] and *TNNI3*[95] mutations. In accordance with those findings, it was reported that the restrictive phenotype (RCM-like HCM) was uncommon in HCM and may represent a poor prognosis form with severe diastolic dysfunction.[96] On the other hand, the difference between RCM-associated mutations and DCM-associated mutations is not clear, but a gene-dose effect could be involved because the RCM-associated *TNNI3* mutation was found in the heterozygous state;[97] whereas the DCM-associated *TNNI3* mutation was found in the homozygous state.[98]

LVNC is a novel cardiomyopathy in which ventricular trabeculations are poorly developed, and mutations in *MYH7*,[99] *CACT*,[100] *LDB3*,[65] *LMNA*,[101] *TAZ*[102] and *DTNA*[48] have been reported (Table 1). The molecular mechanisms of the mutations causing LVNC have not beenelucidated. In a mouse model, deficiency of BMP10 resulted in the LVNC phenotype.[103] BMP10 is a member of the TGFβ family, which is expressed mainly in the heart, and plays a key role in the morphogenesis of the heart.[104] Therefore, LVNC might be a developmental error in hearts carrying mutations in the components of the sarcomere and/or sarcolemma. Interestingly, a rare polymorphism of *BMP10* found in hypertensive DCM decreased binding to Tcap and increased extracellular secretion of BMP10, facilitating the remodeling of hypertensive hearts.[105]

Another primary cardiomyopathy, AVRC, has also recently been well investigated for disease-causing mutations[106] (Table 1). Because the ARVC-associated mutations can be found in the genes for *JUP*,[107] *DSP*,[108] plakophilin-2[109] and desmoglein,[110] they are considered to disrupt cel–cell contacts via desmosomes. *RYR2* mutations have also been reported in ARVC,[111] linking cardiomyopathy to channelopathy (arrhythmia). The promoter variant of TGFβ3 was also reported in ARVC,[112] but its pathological significance remains to be resolved.

Mutations in Cardiomyopathy-Related Diseases

Primary cardiomyopathy includes other related diseases such as mitochondrial cardiomyopathy[113–115] and storage diseases[116–120] affecting the heart. Because mitochondria produce ATP by oxidative phosphorylation, mitochondrial genome mutations cause functional deficits, especially in neuromuscular organs, including the brain, nerves, skeletal and cardiac muscles.[113–115] Mitochondrial cardiomyopathy shows maternal inheritance and is usually found with other neuromuscular disorders. It should be noted that many mitochondrial proteins are encoded by the nuclear genome, so mitochondrial dysfunction is caused not only by mitochondrial genome mutations but also by mutations in the nuclear genome. An example is Barth's syndrome caused by *TAZ* mutations.[90]

Cardiomyopathy with deposition of the glycogen complex in cardiomyocytes is defined as "storage cardiomyopathy". Mutations in genes for the γ subunit of AMP-activated protein kinase (*PRKAG2*),[116–118] α-galactosidase (*GALA*),[119,120] α1,4-glucosidase (*GAA*),[121,122] and lysosome-associated membrane protein-2 (*LAMP2*)[123–125] could cause "storage cardiomyopathy" with a HCM-like phenotype. *PRKAG2* mutations were found in a multiplex family of "HCM associated with WPW syndrome",[116,117] which showed in part a similar clinical course to HCM, but was associated with conduction defects and skeletal muscle involvement.[118] Deficiencies in *GALA*, *GAA*, and *LAMP2* cause Fabry disease,[120] Pompe disease,[122] and Danon disease,[124] respectively, all of which usually manifest with systemic abnormalities, but there are several cases of confined cardiac phenotype, mainly HCM-like phenotype[119,121,123] and partly DCM-like phenotype.[124] The reason for the difference between the systemic type and cardiac restricted type may depend on the residual enzymatic activity expressed by the mutant gene (ie, less severe enzyme defects show the cardiac restricted phenotype). Because *GLA* and *LAMP2* are located on the X chromosome, these diseases are X-linked (ie, patients are usually male); however, a heterozygous female (eg, mother of the patient, carrier of the mutation) may develop partial cardiomyopathy in later life, because X chromosomes in females are randomly inactivated and hence cardiomyocytes in a carrier are a mosaic of *GLA*-expressing cells and non-expressing cells that can develop the disease phenotype.

Conclusions

Genetic causes or gene mutations found in multiplex families and/or patients with hereditary cardiomyopathies have been summarized. Each family or patient has usually only 1 disease-causing mutation, but the primary cardiomyopathy is both clinically and etiologically heterogeneous, even within a specific clinical phenotype (HCM, DCM, RCM, ARVC, and LVNC), which means different causes can result in the same phenotype. These observations suggest that there are common pathways in the pathogenesis of primary cardiomyopathy, such as increased and decreased Ca^{2+} sensitivity in HCM and DCM, respectively. Stiff or loose sarcomeres would also increase and decrease Ca^{2+} sensitivity, respectively. Intervention in these common pathways will be a therapeutic strategy for primary cardiomyopathy caused by different mutations.

Acknowledgments

This work was supported in part by Grant-in-Aids for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan and a research grant for Idiopathic Cardiomyopathy from the Ministry of Health, Labor and Welfare, Japan.

References

1. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, et al. Contemporary definitions and classification of the cardiomyopathies: An American Heart Association Scientific State-

ment from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. *Circulation* 2006; **113:** 1807–1816.
2. Towbin JA, Bowles NE. The failing heart. *Nature* 2002; **415:** 227–233.
3. Ahmad F, Seidman JG, Seidman CE. The genetic basis for cardiac remodeling. *Annu Rev Genomics Hum Genet* 2006; **6:** 185–216.
4. Kushwaha SS, Fallon J, Fuster V. Restrictive cardiomyopathy. *N Engl J Med* 1997; **336:** 267–276.
5. Seidman JG, Seidman C. The genetic basis for cardiomyopathy: From mutation identification to mechanistic paradigms. *Cell* 2001; **104:** 557–567.
6. Goerss JB, Michels VV, Burnett J, Driscoll DJ, Miller F, Rodeheffer R, et al. Frequency of familial dilated cardiomyopathy. *Eur Heart J* 1995; **16**(Suppl O)**:** 2–4.
7. Mestroni L, Rocco C, Gregori D, Sinagra G, Di Lenarda A, Miocic S, et al. Familial dilated cardiomyopathy: Evidence for genetic and phenotypic heterogeneity: Heart Muscle Disease Study Group. *J Am Coll Cardiol* 1999; **34:** 181–190.
8. Geisterfer-Lowrance AA, Kass S, Tanigawa G, Vosberg HP, McKenna W, et al. A molecular basis for familial hypertrophic cardiomyopathy: A beta cardiac myosin heavy chain gene missense mutation. *Cell* 1990; **62:** 999–1006.
9. Nishi H, Kimura A, Harada H, Adachi K, Koga Y, Sasazuki T, et al. Possible gene dose effect of a mutant cardiac beta-myosin heavy chain gene on the clinical expression of familial hypertrophic cardiomyopathy. *Biochem Biophys Res Commun* 1994; **200:** 549–556.
10. Kimura A, Harada H, Park JE, Nishi H, Satoh M, Takahashi M, et al. Mutations in the cardiac troponin I gene associated with hypertrophic cardiomyopathy. *Nat Genet* 1997; **16:** 379–382.
11. Arad M, Seidman JG, Seidman CE. Phenotypic diversity in hypertrophic cardiomyopathy. *Hum Mol Genet* 2002; **11:** 2499–2506.
12. Niimura H, Patton KK, McKenna WJ, Soults J, Maron BJ, Seidman JG, et al. Sarcomere protein gene mutations in hypertrophic cardiomyopathy of the elderly. *Circulation* 2002; **105:** 446–451.
13. Kubo T, Kitaoka H, Okawa M, Matsumura Y, Hitomi N, Yamasaki N, et al. Lifelong left ventricular remodeling of hypertrophic cardiomyopathy caused by a founder frameshift deletion mutation in the cardiac Myosin-binding protein C gene among Japanese. *J Am Coll Cardiol* 2005; **46:** 1737–1743.
14. Sweeney HL, Straceski AJ, Leinwand LA, Tikunov BA, Faust L. Heterologous expression of a cardiomyopathic myosin that is defective in its actin interaction. *J Biol Chem* 1994; **269:** 1603–1605.
15. Thierfelder L, Watkins H, MacRae C, Lamas R, McKenna W, Vosberg HP, et al. Alpha-tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: A disease of the sarcomere. *Cell* 1994; **77:** 701–712.
16. Yanaga F, Morimoto S, Ohtsuki I. Ca^{2+} sensitization and potentiation of the maximum level of myofibrillar ATPase activity caused by mutations of troponin T found in familial hypertrophic cardiomyopathy. *J Biol Chem* 1999; **274:** 8806–8812.
17. Bottinelli R, Coviello DA, Redwood CS, Pellegrino MA, Maron BJ, Spirito P, et al. A mutant tropomyosin that causes hypertrophic cardiomyopathy is expressed in vivo and associated with an increased calcium sensitivity. *Circ Res* 1998; **82:** 106–115.
18. Elliott K, Watkins H, Redwood CS. Altered regulatory properties of human cardiac troponin I mutants that cause hypertrophic cardiomyopathy. *J Biol Chem* 2000; **275:** 22069–22074.
19. Witt CC, Gerull B, Davies MJ, Centner T, Linke WA, Thierfelder L. Hypercontractile properties of cardiac muscle fibers in a knock-in mouse model of cardiac myosin-binding protein-C. *J Biol Chem* 2001; **276:** 5353–5359.
20. Roopnarine O. Mechanical defects of muscle fibers with myosin light chain mutants that cause cardiomyopathy. *Biophys J* 2003; **84:** 2440–2449.
21. Tyska MJ, Hayes E, Giewat M, Seidman CE, Seidman JG, Warshaw DM. Single-molecule mechanics of R403Q cardiac myosin isolated from the mouse model of familial hypertrophic cardiomyopathy. *Circ Res* 2000; **86:** 737–744.
22. Satoh M, Takahashi M, Sakamoto T, Hiroe M, Marumo F, Kimura A. Structural analysis of the titin gene in hypertrophic cardiomyopathy: Identification of a novel disease gene. *Biochem Biophys Res Commun* 1999; **262:** 411–417.
23. Hayashi T, Arimura T, Itoh-Satoh M, Ueda K, Hohda S, Inagaki N, et al. Tcap gene mutations in hypertrophic cardiomyopathy and dilated cardiomyopathy. *J Am Coll Cardiol* 2004; **44:** 2192–2201.
24. Cazorla O, Wu Y, Irving TC, Granzier H. Titin-based modulation of calcium sensitivity of active tension in mouse skinned cardiac myocytes. *Circ Res* 2001; **88:** 1028–1035.
25. Fujita H, Labeit D, Gerull B, Labeit S, Granzier HL. Titin isoform-dependent effect of calcium on passive myocardial tension. *Am J Physiol Heart Circ Physiol* 2004; **287:** H2528–H2534.
26. Fuchs F, Martyn DA. Length-dependent Ca(2+) activation in cardiac muscle: Some remaining questions. *J Muscle Res Cell Motil* 2005; **26:** 199–212.
27. Geier C, Perrot A, Ozcelik C, Binner P, Counsell D, Hoffmann K, et al. Mutations in the human muscle LIM protein gene in families with hypertrophic cardiomyopathy. *Circulation* 2003; **107:** 1390–1395.
28. Gehmlich K, Geier C, Osterziel KJ, Van der Ven PF, Fürst DO. Decreased interactions of mutant muscle LIM protein (MLP) with N-RAP and alpha-actinin and their implication for hypertrophic cardiomyopathy. *Cell Tissue Res* 2004; **317:** 129–136.
29. Mohapatra B, Jimenez S, Lin JH, Bowles KR, Coveler KJ, Marx JG, et al. Mutations in the muscle LIM protein and alpha-actinin-2 genes in dilated cardiomyopathy and endocardial fibroelastosis. *Mol Genet Metab* 2003; **80:** 207–215.
30. Hayashi T, Arimura T, Ueda K, Shibata H, Hohda S, Takahashi M, et al. Identification and functional analysis of a caveolin-3 mutation associated with familial hypertrophic cardiomyopathy. *Biochem Biophys Res Commun* 2004; **313:** 178–184.
31. Vasile VC, Ommen SR, Edwards WD, Ackerman MJ. A missense mutation in a ubiquitously expressed protein, vinculin, confers susceptibility to hypertrophic cardiomyopathy. *Biochem Biophys Res Commun* 2006; **345:** 998–1003.
32. Wang X, Osinska H, Klevitsky R, Gerdes AM, Nieman M, Lorenz J, et al. Expression of R120G-alphaB-crystallin causes aberrant desmin and alphaB-crystallin aggregation and cardiomyopathy in mice. *Circ Res* 2001; **89:** 84–91.
33. Landstrom AP, Weisleder N, Batalden KB, Bos JM, Tester DJ, Ommen SR, et al. Mutations in JPH2-encoded junctophilin-2 associated with hypertrophic cardiomyopathy in humans. *J Mol Cell Cardiol* 2007; **42:** 1026–1035.
34. Arimura T, Matsumoto Y, Okazaki O, Hayashi T, Takahashi M, Inagaki N, et al. Structural analysis of obscurin gene in hypertrophic cardiomyopathy. *Biochem Biophys Res Commun* 2007; **362:** 281–287.
35. Rajasekaran NS, Connell P, Christians ES, Yan LJ, Taylor RP, Orosz A, et al. Human alpha B-crystallin mutation causes oxido-reductive stress and protein aggregation cardiomyopathy in mice. *Cell* 2007; **130:** 427–439.
36. Matsumoto Y, Hayashi T, Inagaki N, Takahashi M, Hiroi S, Nakamura T, et al. Functional analysis of titin/connectin N2-B mutations found in cardiomyopathy. *J Muscle Res Cell Motil* 2005; **26:** 367–374.
37. Inagaki N, Hayashi T, Arimura T, Koga Y, Takahashi M, Shibata H, et al. Alpha B-crystallin mutation in dilated cardiomyopathy. *Biochem Biophys Res Commun* 2006; **342:** 379–386.
38. Kikuchi T, Oka N, Koga A, Miyazaki H, Ohmura H, Imaizumi T. Behavior of caveolae and caveolin-3 during the development of myocyte hypertrophy. *J Cardiovasc Pharmacol* 2005; **45:** 204–210.
39. Koga A, Oka N, Kikuchi T, Miyazaki H, Kato S, Imaizumi T. Adenovirus-mediated overexpression of caveolin-3 inhibits rat cardiomyocyte hypertrophy. *Hypertension* 2003; **42:** 213–219.
40. Young P, Ehler E, Gautel M. Obscurin, a giant sarcomeric Rho guanine nucleotide exchange factor protein involved in sarcomere assembly. *J Cell Biol* 2001; **154:** 123–136.
41. Towbin JA, Hejtmancik JF, Brink P, Gelb B, Zhu XM, Chamberlain JS, et al. X-linked dilated cardiomyopathy: Molecular genetic evidence of linkage to the Duchenne muscular dystrophy (dystrophin) gene at the Xp21 locus. *Circulation* 1993; **87:** 1854–1865.
42. Cohen N, Muntoni F. Multiple pathogenetic mechanisms in X linked dilated cardiomyopathy. *Heart* 2004; **90:** 835–841.
43. Muntoni F, Torelli S, Ferlini A. Dystrophin and mutations: One gene, several proteins, multiple phenotypes. *Lancet Neurol* 2003; **2:** 731–740.
44. Finsterer J, Stöllberger C. The heart in human dystrophinopathies. *Cardiology* 2003; **99:** 1–19.
45. Lapidos KA, Kakkar R, McNally EM. The dystrophin glycoprotein complex: Signaling strength and integrity for the sarcolemma. *Circ Res* 2004; **94:** 1023–1031.
46. Tsubata S, Bowles KR, Vatta M, Zintz C, Titus J, Muhonen L, et al. Mutations in the human delta-sarcoglycan gene in familial and sporadic dilated cardiomyopathy. *J Clin Invest* 2000; **106:** 655–662.
47. Knöll R, Postel R, Wang J, Krätzner R, Hennecke G, Vacaru AM, et al. Laminin-alpha4 and integrin-linked kinase mutations cause human cardiomyopathy via simultaneous defects in cardiomyocytes and endothelial cells. *Circulation* 2007; **116:** 515–525.

48. Ichida F, Tsubata S, Bowles KR, Haneda N, Uese K, Miyawaki T, et al. Novel gene mutations in patients with left ventricular noncompaction or Barth syndrome. *Circulation* 2001; **103:** 1256–1263.
49. Towbin JA, Bowles NE. Genetic abnormalities responsible for dilated cardiomyopathy. *Curr Cardiol Rep* 2000; **2:** 475–480.
50. Olson TM, Michels VV, Thibodeau SN, Tai YS, Keating MT. Actin mutations in dilated cardiomyopathy, a heritable form of heart failure. *Science* 1998; **280:** 750–752.
51. Mogensen J, Klausen IC, Pedersen AK, Egeblad H, Bross P, Kruse TA, et al. Alpha-cardiac actin is a novel disease gene in familial hypertrophic cardiomyopathy. *J Clin Invest* 1999; **103:** R39–R43.
52. Vang S, Corydon TJ, Børglum AD, Scott MD, Frydman J, Mogensen J, et al. Actin mutations in hypertrophic and dilated cardiomyopathy cause inefficient protein folding and perturbed filament formation. *FEBS J* 2005; **272:** 2037–2049.
53. Kamisago M, Sharma SD, DePalma SR, Solomon S, Sharma P, McDonough B, et al. Mutations in sarcomere protein genes as a cause of dilated cardiomyopathy. *N Engl J Med* 2000; **343:** 1688–1696.
54. Morimoto S, Lu QW, Harada K, Takahashi-Yanaga F, Minakami R, Ohta M, et al. Ca(2+)-desensitizing effect of a deletion mutation Delta K210 in cardiac troponin T that causes familial dilated cardiomyopathy. *Proc Natl Acad Sci USA* 2002; **99:** 913–918.
55. Chang AN, Potter JD. Sarcomeric protein mutations in dilated cardiomyopathy. *Heart Fail Rev* 2005; **10:** 225–235.
56. Itoh-Satoh M, Hayashi T, Nishi H, Koga Y, Arimura T, Koyanagi T, et al. Titin mutations as the molecular basis for dilated cardiomyopathy. *Biochem Biophys Res Commun* 2002; **291:** 385–393.
57. Knöll R, Hoshijima M, Hoffman HM, Person V, Lorenzen-Schmidt I, Bang ML, et al. The cardiac mechanical stretch sensor machinery involves a Z disc complex that is defective in a subset of human dilated cardiomyopathy. *Cell* 2002; **111:** 943–955.
58. Zhou Q, Ruiz-Lozano P, Martone ME, Chen J. Cypher, a striated muscle-restricted PDZ and LIM domain-containing protein, binds to alpha-actinin-2 and protein kinase C. *J Biol Chem* 1999; **274:** 19807–19813.
59. Frey N, Richardson JA, Olson EN. Calsarcins, a novel family of sarcomeric calcineurin-binding proteins. *Proc Natl Acad Sci USA* 2000; **97:** 14632–14637.
60. Wilkins BJ, Molkentin JD. Calcium-calcineurin signaling in the regulation of cardiac hypertrophy. *Biochem Biophys Res Commun* 2004; **322:** 1178–1191.
61. Heineke J, Molkentin JD. Regulation of cardiac hypertrophy by intracellular signalling pathways. *Nat Rev Mol Cell Biol* 2006; **7:** 589–600.
62. Heineke J, Ruetten H, Willenbockel C, Gross SC, Naguib M, Schaefer A, et al. Attenuation of cardiac remodeling after myocardial infarction by muscle LIM protein-calcineurin signaling at the sarcomeric Z-disc. *Proc Natl Acad Sci USA* 2005; **102:** 1655–1660.
63. Arimura T, Hayashi T, Terada H, Lee SY, Zhou Q, Takahashi M, et al. A Cypher/ZASP mutation associated with dilated cardiomyopathy alters the binding affinity to protein kinase C. *J Biol Chem* 2004; **279:** 6746–6752.
64. Vatta M, Mohapatra B, Jimenez S, Sanchez X, Faulkner G, Perles Z, et al. Mutations in Cypher/ZASP in patients with dilated cardiomyopathy and left ventricular non-compaction. *J Am Coll Cardiol* 2003; **42:** 2014–2027.
65. Li D, Tapscoft T, Gonzalez O, Burch PE, Quiñones MA, Zoghbi WA, et al. Desmin mutation responsible for idiopathic dilated cardiomyopathy. *Circulation* 1999; **100:** 461–464.
66. Olson TM, Illenberger S, Kishimoto NY, Huttelmaier S, Keating MT, Jockusch BM. Metavinculin mutations alter actin interaction in dilated cardiomyopathy. *Circulation* 2002; **105:** 431–437.
67. Taylor MR, Slavov D, Ku L, Di Lenarda A, Sinagra G, Carniel E, et al. Prevalence of desmin mutations in dilated cardiomyopathy. *Circulation* 2007; **115:** 1244–1251.
68. Duboscq-Bidot L, Xu P, Charron P, Neyroud N, Dilanian G, Millaire A, et al. Mutations in the Z-band protein myopalladin gene and idiopathic dilated cardiomyopathy. *Cardiovasc Res* 2008; **77:** 118–125.
69. Fatkin D, MacRae C, Sasaki T, Wolff MR, Porcu M, Frenneaux M, et al. Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. *N Engl J Med* 1999; **341:** 1715–1724.
70. Bonne G, Di Barletta MR, Varnous S, Bécane HM, Hammouda EH, Merlini L, et al. Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. *Nat Genet* 1999; **21:** 285–288.
71. Bione S, Small K, Aksmanovic VM, D'Urso M, Ciccodicola A, Merlini L, et al. Identification of new mutations in the Emery-Dreifuss muscular dystrophy gene and evidence for genetic heterogeneity of the disease. *Hum Mol Genet* 1995; **4:** 1859–1863.
72. Morris GE, Manilal S. Heart to heart: From nuclear proteins to Emery-Dreifuss muscular dystrophy. *Hum Mol Genet* 1999; **8:** 1847–1851.
73. Sylvius N, Tesson F. Lamin A/C and cardiac diseases. *Curr Opin Cardiol* 2006; **21:** 159–165.
74. Bienengraeber M, Olson TM, Selivanov VA, Kathmann EC, O'Cochlain F, Gao F, et al. ABCC9 mutations identified in human dilated cardiomyopathy disrupt catalytic KATP channel gating. *Nat Genet* 2004; **36:** 382–387.
75. McNair WP, Ku L, Taylor MR, Fain PR, Dao D, Wolfel E, et al. SCN5A mutation associated with dilated cardiomyopathy, conduction disorder, and arrhythmia. *Circulation* 2004; **110:** 2163–2167.
76. Lehnart SE, Ackerman MJ, Benson DW Jr, Brugada R, Clancy CE, Donahue JK, et al. Inherited arrhythmias: A National Heart, Lung, and Blood Institute and Office of Rare Diseases workshop consensus report about the diagnosis, phenotyping, molecular mechanisms, and therapeutic approaches for primary cardiomyopathies of gene mutations affecting ion channel function. *Circulation* 2007; **116:** 2325–2345.
77. Arimura T, Hayashi T, Matsumoto Y, Shibata H, Hiroi S, Nakamura T, et al. Structural analysis of four and half LIM protein-2 in dilated cardiomyopathy. *Biochem Biophys Res Commun* 2007; **357:** 162–167.
78. Johannessen M, Møller S, Hansen T, Moens U, Van Ghelue M. The multifunctional roles of the four-and-a-half-LIM only protein FHL2. *Cell Mol Life Sci* 2006; **63:** 268–284.
79. Hoshijima M, Knöll R, Pashmforoush M, Chien KR. Reversal of calcium cycling defects in advanced heart failure toward molecular therapy. *J Am Coll Cardiol* 2006; **48:** A15–A23.
80. Schmitt JP, Kamisago M, Asahi M, Li GH, Ahmad F, Mende U, et al. Dilated cardiomyopathy and heart failure caused by a mutation in phospholamban. *Science* 2003; **299:** 1410–1413.
81. Haghighi K, Kolokathis F, Gramolini AO, Waggoner JR, Pater L, Lynch RA, et al. A mutation in the human phospholamban gene, deleting arginine 14, results in lethal, hereditary cardiomyopathy. *Proc Natl Acad Sci USA* 2006; **103:** 1388–1393.
82. Chiu C, Tebo M, Ingles J, Yeates L, Arthur JW, Lind JM, et al. Genetic screening of calcium regulation genes in familial hypertrophic cardiomyopathy. *J Mol Cell Cardiol* 2007; **43:** 337–343.
83. Koss KL, Grupp IL, Kranias EG. The relative phospholamban and SERCA2 ratio: A critical determinant of myocardial contractility. *Basic Res Cardiol* 1997; **92**(Suppl 1)**:** 17–24.
84. Minamisawa S, Hoshijima M, Chu G, Ward CA, Frank K, Gu Y, et al. Chronic phospholamban-sarcoplasmic reticulum calcium ATPase interaction is the critical calcium cycling defect in dilated cardiomyopathy. *Cell* 1999; **99:** 313–322.
85. Hoshijima M, Ikeda Y, Iwanaga Y, Minamisawa S, Date MO, Gu Y, et al. Chronic suppression of heart-failure progression by a pseudophosphorylated mutant of phospholamban via in vivo cardiac rAAV gene delivery. *Nat Med* 2002; **8:** 864–871.
86. Nigro V, Okazaki Y, Belsito A, Piluso G, Matsuda Y, Politano L, et al. Identification of the Syrian hamster cardiomyopathy gene. *Hum Mol Genet* 1997; **6:** 601–607.
87. Minamisawa S, Sato Y, Tatsuguchi Y, Fujino T, Imamura S, Uetsuka Y, et al. Mutation of the phospholamban promoter associated with hypertrophic cardiomyopathy. *Biochem Biophys Res Commun* 2003; **304:** 1–4.
88. Medin M, Hermida-Prieto M, Monserrat L, Laredo R, Rodriguez-Rey JC, Fernandez X, et al. Mutational screening of phospholamban gene in hypertrophic and idiopathic dilated cardiomyopathy and functional study of the PLN-42 C>G mutation. *Eur J Heart Fail* 2007; **9:** 37–43.
89. Kadambi VJ, Ponniah S, Harrer JM, Hoit BD, Dorn GW 2nd, Walsh RA, et al. Cardiac-specific overexpression of phospholamban alters calcium kinetics and resultant cardiomyocyte mechanics in transgenic mice. *J Clin Invest* 1996; **97:** 533–539.
90. Barth PG, Valianpour F, Bowen VM, Lam J, Duran M, Vaz FM, et al. X-linked cardioskeletal myopathy and neutropenia (Barth syndrome): An update. *Am J Med Genet A* 2004; **126:** 349–354.
91. Murakami T, Hayashi YK, Noguchi S, Ogawa M, Nonaka I, Tanabe Y, et al. Fukutin gene mutations cause dilated cardiomyopathy with minimal muscle weakness. *Ann Neurol* 2006; **60:** 597–602.
92. Norgett EE, Hatsell SJ, Carvajal-Huerta L, Cabezas JC, Common J, Purkis PE, et al. Recessive mutation in desmoplakin disrupts desmoplakin-intermediate filament interactions and causes dilated cardiomyopathy, woolly hair and keratoderma. *Hum Mol Genet*

2000; **9:** 2761–2766.
93. Uzumcu A, Norgett EE, Dindar A, Uyguner O, Nisli K, Kayserili H, et al. Loss of desmoplakin isoform I causes early onset cardiomyopathy and heart failure in a Naxos-like syndrome. *J Med Genet* 2006; **43:** e5.
94. Pinto JR, Parvatiyar MS, Jones MA, Liang J, Potter JD. A troponin T mutation that causes infantile restrictive cardiomyopathy increases Ca^{2+} sensitivity of force development and impairs the inhibitory properties of troponin. *J Biol Chem* 2008; **283:** 2156–2166.
95. Yumoto F, Lu QW, Morimoto S, Tanaka H, Kono N, Nagata K, et al. Drastic Ca2+ sensitization of myofilament associated with a small structural change in troponin I in inherited restrictive cardiomyopathy. *Biochem Biophys Res Commun* 2005; **338:** 1519–1526.
96. Kubo T, Gimeno JR, Bahl A, Steffensen U, Steffensen M, Osman E, et al. Prevalence, clinical significance, and genetic basis of hypertrophic cardiomyopathy with restrictive phenotype. *J Am Coll Cardiol* 2007; **49:** 2419–2426.
97. Mogensen J, Kubo T, Duque M, Uribe W, Shaw A, Murphy R, et al. Idiopathic restrictive cardiomyopathy is part of the clinical expression of cardiac troponin I mutations. *J Clin Invest* 2003; **111:** 209–216.
98. Murphy RT, Mogensen J, Shaw A, Kubo T, Hughes S, McKenna WJ. Novel mutation in cardiac troponin I in recessive idiopathic dilated cardiomyopathy. *Lancet* 2004; **363:** 371–372.
99. Budde BS, Binner P, Waldmüller S, Höhne W, Blankenfeldt W, Hassfeld S, et al. Noncompaction of the ventricular myocardium is associated with a de novo mutation in the beta-myosin heavy chain gene. *PLoS ONE* 2007; **2:** e1362.
100. Monserrat L, Hermida-Prieto M, Fernandez X, Rodríguez I, Dumont C, Cazón L, et al. Mutation in the alpha-cardiac actin gene associated with apical hypertrophic cardiomyopathy, left ventricular non-compaction, and septal defects. *Eur Heart J* 2007; **28:** 1953–1961.
101. Hermida-Prieto M, Monserrat L, Castro-Beiras A, Laredo R, Soler R, Peteiro J, et al. Familial dilated cardiomyopathy and isolated left ventricular noncompaction associated with lamin A/C gene mutations. *Am J Cardiol* 2004; **94:** 50–54.
102. Bleyl SB, Mumford BR, Thompson V, Carey JC, Pysher TJ, Chin TK, et al. Neonatal, lethal noncompaction of the left ventricular myocardium is allelic with Barth syndrome. *Am J Hum Genet* 1997; **61:** 868–872.
103. Chen H, Shi S, Acosta L, Li W, Lu J, Bao S, et al. BMP10 is essential for maintaining cardiac growth during murine cardiogenesis. *Development* 2004; **131:** 2219–2231.
104. Neuhaus H, Rosen V, Thies RS. Heart specific expression of mouse BMP-10 a novel member of the TGF-beta superfamily. *Mech Dev* 1999; **80:** 181–184.
105. Nakano N, Hori H, Abe M, Shibata H, Arimura T, Sasaoka T, et al. Interaction of BMP10 with Tcap may modulate the course of hypertensive cardiac hypertrophy. *Am J Physiol Heart Circ Physiol* 2007; **293:** H3396–H3403.
106. van Tintelen JP, Hofstra RM, Wiesfeld AC, van den Berg MP, Hauer RN, Jongbloed JD. Molecular genetics of arrhythmogenic right ventricular cardiomyopathy: Emerging horizon? *Curr Opin Cardiol* 2007; **22:** 185–192.
107. McKoy G, Protonotarios N, Crosby A, Tsatsopoulou A, Anastasakis A, Coonar A, et al. Identification of a deletion in plakoglobin in arrhythmogenic right ventricular cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease). *Lancet* 2000; **355:** 2119–2124.
108. Alcalai R, Metzger S, Rosenheck S, Meiner V, Chajek-Shaul T. A recessive mutation in desmoplakin causes arrhythmogenic right ventricular dysplasia, skin disorder, and woolly hair. *J Am Coll Cardiol* 2003; **42:** 319–327.
109. Gerull B, Heuser A, Wichter T, Paul M, Basson CT, McDermott DA, et al. Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy. *Nat Genet* 2004; **36:** 1162–1164.
110. Pilichou K, Nava A, Basso C, Beffagna G, Bauce B, Lorenzon A, et al. Mutations in desmoglein-2 gene are associated with arrhythmogenic right ventricular cardiomyopathy. *Circulation* 2006; **113:** 1171–1179.
111. Tiso N, Stephan DA, Nava A, Bagattin A, Devaney JM, Stanchi F, et al. Identification of mutations in the cardiac ryanodine receptor gene in families affected with arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2). *Hum Mol Genet* 2001; **10:** 189–194.
112. Beffagna G, Occhi G, Nava A, Vitiello L, Ditadi A, Basso C, et al. Regulatory mutations in transforming growth factor-beta3 gene cause arrhythmogenic right ventricular cardiomyopathy type 1. *Cardiovasc Res* 2005; **65:** 366–373.
113. Antozzi C, Zeviani M. Cardiomyopathies in disorders of oxidative metabolism. *Cardiovasc Res* 1997; **35:** 184–199.
114. Wallace DC. Mitochondrial defects in cardiomyopathy and neuromuscular disease. *Am Heart J* 2000; **139:** S70–S85.
115. DiMauro S. Mitochondrial myopathies. *Curr Opin Rheumatol* 2006; **18:** 636–641.
116. Gollob MH, Green MS, Tang AS, Gollob T, Karibe A, Ali Hassan AS, et al. Identification of a gene responsible for familial Wolff-Parkinson-White syndrome. *N Engl J Med* 2001; **344:** 1823–1831.
117. Blair E, Redwood C, Ashrafian H, Oliveira M, Broxholme J, Kerr B, et al. Mutations in the gamma (2) subunit of AMP-activated protein kinase cause familial hypertrophic cardiomyopathy: Evidence for the central role of energy compromise in disease pathogenesis. *Hum Mol Genet* 2001; **10:** 1215–1220.
118. Murphy RT, Mogensen J, McGarry K, Bahl A, Evans A, Osman E, et al. Adenosine monophosphate-activated protein kinase disease mimics hypertrophic cardiomyopathy and Wolff-Parkinson-White syndrome: Natural history. *J Am Coll Cardiol* 2005; **45:** 922–930.
119. Nakao S, Takenaka T, Maeda M, Kodama C, Tanaka A, Tahara M, et al. An atypical variant of Fabry's disease in men with left ventricular hypertrophy. *N Engl J Med* 1995; **333:** 288–293.
120. Desnick RJ, Brady R, Barranger J, Collins AJ, Germain DP, Goldman M, et al. Fabry disease, an under-recognized multisystemic disorder: Expert recommendations for diagnosis, management, and enzyme replacement therapy. *Ann Intern Med* 2003; **138:** 338–346.
121. Arad M, Maron BJ, Gorham JM, Johnson WH Jr, Saul JP, Perez-Atayde AR, et al. Glycogen storage diseases presenting as hypertrophic cardiomyopathy. *N Engl J Med* 2005; **352:** 362–372.
122. Raben N, Plotz P, Byrne BJ. Acid alpha-glucosidase deficiency (glycogenosis type II, Pompe disease). *Curr Mol Med* 2002; **2:** 145–166.
123. Yang Z, McMahon CJ, Smith LR, Bersola J, Adesina AM, Breinholt JP, et al. Danon disease as an underrecognized cause of hypertrophic cardiomyopathy in children. *Circulation* 2005; **112:** 1612–1617.
124. Horváth J, Ketelsen UP, Geibel-Zehender A, Boehm N, Olbrich H, Korinthenberg R, et al. Identification of a novel LAMP2 mutation responsible for X-chromosomal dominant Danon disease. *Neuropediatrics* 2003; **34:** 270–273.
125. Taylor MR, Ku L, Slavov D, Cavanaugh J, Boucek M, Zhu X, et al. Danon disease presenting with dilated cardiomyopathy and a complex phenotype. *J Hum Genet* 2007; **52:** 830–835.