

REVIEW
Translational Physiology

Understanding the molecular basis of cardiomyopathy

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Abstract

Inherited cardiomyopathies are a major cause of mortality and morbidity worldwide and can be caused by mutations in a wide range of proteins located in different cellular compartments. The present review is based on Dr. Ju Chen's 2021 Robert M. Berne Distinguished Lectureship of the American Physiological Society Cardiovascular Section, in which he provided an overview of the current knowledge on the cardiomyopathy-associated proteins that have been studied in his laboratory. The review provides a general summary of the proteins in different compartments of cardiomyocytes associated with cardiomyopathies, with specific focus on the proteins that have been studied in Dr. Chen's laboratory.

cardiomyopathy; genetics; murine models; protein mutations; translational research

INTRODUCTION

The heart is a complex organ composed of cardiomyocytes as well as other cell types, such as endothelial cells, vascular smooth muscle cells, fibroblasts, pericytes, immune-related cells, and others (1, 2). Cardiomyocytes are the contractile cells of the myocardium, allowing the heart to pump. The structural organization of the cardiomyocyte is depicted in Fig. 1. The basic contractile unit is the sarcomere, which is made up of thick and thin filaments sliding over each other during contraction (3, 4). Sarcomeres are organized into myofilaments that are functionally connected at intercalated disks, which join individual cardiomyocytes together, allowing the cardiomyocytes to contract as a single coordinated unit and permitting mechanical and electrical coupling between cells (5–7). The sarcolemma, i.e., the cardiomyocyte plasma membrane, contains deep invaginations called t-tubes (transverse tubules), which allow for rapid transmission of the action potential to the interior of the cell to induce muscle contraction in a process called excitation-contraction coupling (8–10). The action potential is induced by pacemaker cells in the sinoatrial and atrioventricular nodes, which causes an influx of calcium through calcium channels in the t-tubules, triggering synchronized calcium release from the nearby sarcoplasmic reticulum (SR), the calcium storage unit of the cell, to induce muscle contraction. Cytosolic calcium is subsequently pumped back into the SR, causing relaxation. To meet the high energy demand of the continuously contracting cardiomyocytes, they contain a high

number of mitochondria (11). A network of microtubules, intermediate filaments, and nonsarcomeric actin laterally links adjacent myofibrils, associates nuclei to the sarcomere, and connects myofibrils to the sarcolemma and the extracellular matrix at the costamere, providing a structural framework for the cell (12, 13). Furthermore, the cytoskeletal network mediates the transport of proteins and organelles and plays an important role in mechanosensing and signal transduction.

Mutations in structural or regulatory proteins of the cardiomyocyte can lead to cardiomyopathy, which is a heterogeneous disease defined as “a myocardial disorder in which the heart is structurally and functionally abnormal in the absence of coronary artery disease, hypertension, valvular disease and congenital heart disease sufficient to cause the observed myocardial abnormality” (14). Cardiomyopathies can be divided into hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), arrhythmogenic cardiomyopathy (ACM), and left ventricular noncompaction (LVNC), which can be further classified into genetic and acquired forms (15).

HCM affects ~1 in 500 and is the leading cause of sudden cardiac death in young athletes (16, 17). HCM is characterized by left ventricular (LV) hypertrophy in the absence of hemodynamic stresses (e.g., hypertension, aortic valve stenosis) or systemic diseases, such as amyloidosis and glycogen storage disease (reviewed in Refs. 18, 19). Patients typically show preserved systolic function but impaired LV relaxation (i.e., diastolic dysfunction), which may eventually develop into LV

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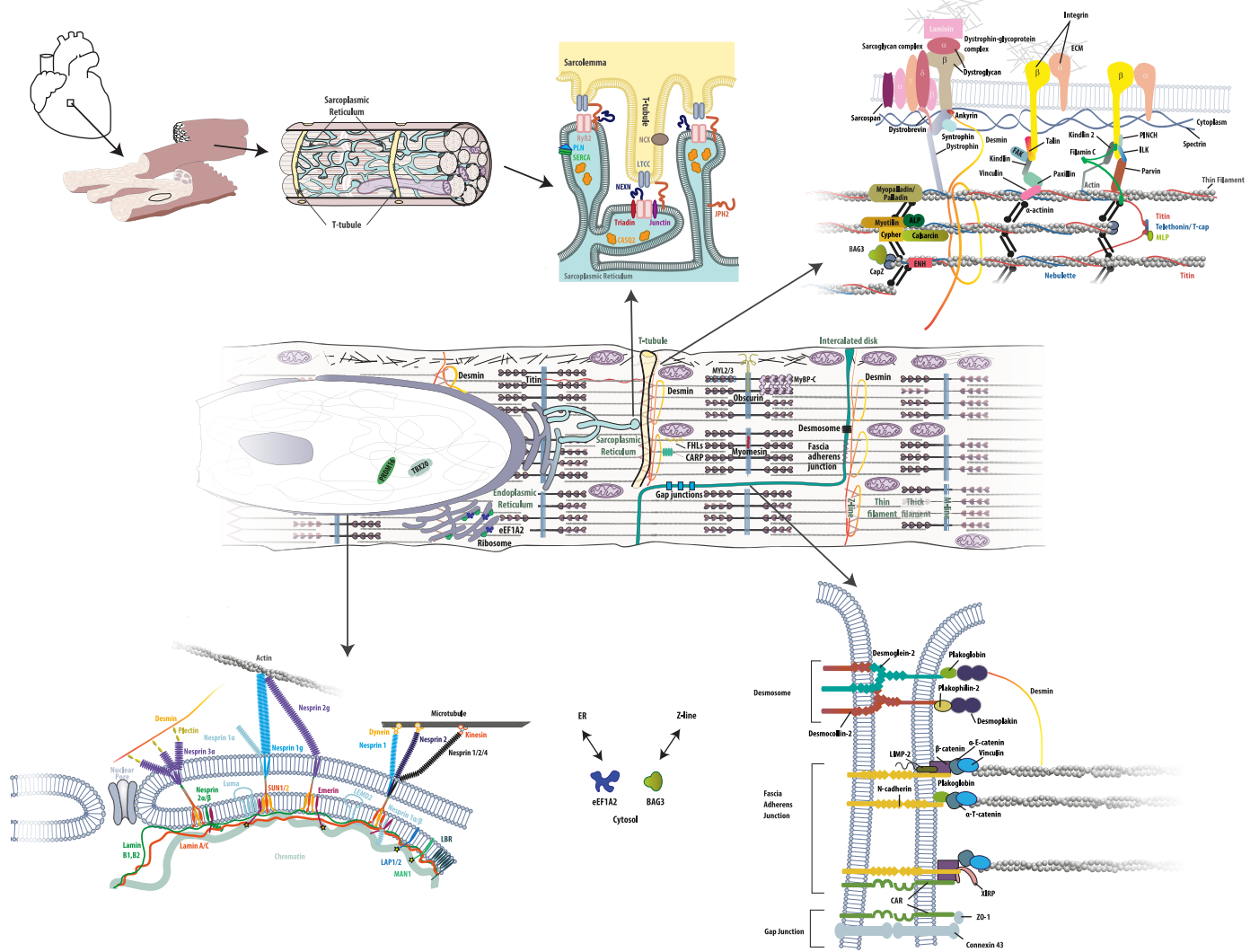


Figure 1. Schematic representation of cardiomyocyte structure, including key proteins associated with human cardiomyopathy. Cardiomyopathy-associated proteins studied in Dr. Ju Chen’s laboratory (listed in Table 1) are located in different compartments of the cardiomyocyte, including the sarcomere, intercalated disk (ICD), sarcoplasmic reticulum (SR), costamere, nucleus, and cytosol. ALP, actinin-associated lim protein; BAG3, BCL2-associated athanogene 3; CAR, coxsackievirus and adenovirus receptor; CARP, cardiac ankyrin repeat; CASQ2, calsequestrin 2; ECM, extracellular matrix; eEF1A2, eukaryotic elongation factor 1 alpha 2; ENH, Enigma homolog protein; ER, endoplasmic reticulum; FAK, focal adhesion kinase; ILK, integrin linked kinase; FHLS, four and a half LIM proteins; ILK, integrin-linked kinase; JPH2, junctophilin 2; LAMP1/2, lamina-associated polypeptide 1 and 2; LBR, lamin B receptor; LEMD2, LEM domain nuclear envelope protein 2; LIMP-2, lysosomal integral membrane protein-2; LTCC, L-type calcium channel; MLP, muscle LIM protein; MYL2/3, myosin light chain 2/3; MYBP-C, myosin binding protein-C; NCX, sodium/calcium exchanger; PLN, phospholamban; PRDM16, PR/SET domain 16; RyR2, ryanodine receptor 2; SERCA, SR calcium ATPase; SUN1/2, Sad1 and UNC84 domain-containing 1 and 2; TBX20, T-box transcription factor 20; T-cap, titin cap; XIRP, xin actin-binding repeat containing; ZO-1, zonula occludens-1.

dilation, wall thinning, and systolic dysfunction. However, the clinical manifestation is very variable, ranging from asymptomatic LV hypertrophy to severe heart failure or sudden cardiac death. At the histological level, cardiomyocyte hypertrophy, myofibrillar disarray, and interstitial fibrosis are typical features. HCM is in most cases caused by autosomal dominant mutations in genes encoding sarcomeric proteins, which can be identified in ~60% of clinical cases (18). A common molecular mechanism leading to HCM is enhanced calcium sensitivity and affinity of the myofilament as well as inefficient ATP utilization for tension generation, resulting in a higher energy demand and consequent energetic inefficiency (reviewed in Ref. 20).

DCM is characterized by progressive ventricular dilation and systolic dysfunction in the absence of abnormal loading

conditions (e.g., hypertension, coronary artery disease, valvular disease) (reviewed in Refs. 21–24) and has an estimated prevalence of 1 in 250 (24). The age of onset ranges from newborn to old age, although most patients are diagnosed between 20 and 50 yr of age. The clinical manifestation varies from asymptomatic to severe and may include heart failure, arrhythmia, thromboembolism, conduction defects, and sudden cardiac death. DCM can be caused by a wide range of conditions, such as inflammatory conditions (e.g., myocarditis, autoimmune disease), metabolic disorders (e.g., hyperthyroidism), and toxins (e.g., alcohol, chemotoxins, drugs) (22). Furthermore, 30–50% of DCM cases are familial (25–27), and causative gene mutations have been identified in >100 genes encoding proteins of various cellular compartments, including

the sarcomere, intercalated disk (ICD), costamere, SR, mitochondria, sarcolemma, and nuclear envelope (reviewed in Ref. 21). The inheritance is mostly autosomal dominant, but autosomal recessive, X-linked, and mitochondrial inheritance have also been described. In contrast to HCM, reduced myofilament calcium sensitivity is commonly associated with DCM. Furthermore, molecular mechanisms associated with DCM include impaired force generation and transmission, altered myofilament calcium handling, ion channel dysfunction, defective mechanosensing, myocardial energy deficit, and structural changes in the sarcomere, cytoskeleton, and/or nucleus (21).

RCM is a rare form of cardiomyopathy characterized by increased ventricular stiffness, resulting in impaired ventricular filling and consequent abnormal relaxation of the ventricles and diastolic dysfunction (reviewed in Ref. 28). Moreover, the atria are often enlarged because of increased end-diastolic pressure in the ventricles, whereas wall thickness and ventricular volume typically remain normal until advanced stages of the disease. The prevalence of RCM is unknown, but it has been estimated to account for <5% of cardiomyopathies. Among the cardiomyopathies, RCM has the poorest prognosis, especially in children, where the mortality is as high as 50% within the first 2 yr after diagnosis (29). In particular, RCM is associated with an increased risk of arrhythmias and sudden cardiac death. Common causes of RCM are infiltrative disease (e.g., amyloidosis, sarcoidosis), lysosomal and glycogen storage disorders (e.g., Fabry disease, hemochromatosis, glycogen storage disease), endomyocardial fibrosis, and cancer treatments (anthracycline, radiation) (30). Furthermore, RCM can be familial, mostly caused by autosomal dominant mutations in genes encoding sarcomeric and sarcomere-associated proteins (28). Many RCM-associated mutations have been associated with increased myofilament calcium sensitivity, resulting in delayed relaxation and increased energy consumption, whereas others cause protein aggregation, likely as a result of impaired protein quality control.

LVNC is characterized by prominent LV trabeculations, deep intratrabecular recesses, and a two-layered LV wall composed of a thin compacted outer (epicardial) layer and a thicker noncompacted inner (endothelial) layer, giving the LV a spongy appearance (reviewed in Refs. 31–33). Pathological LVNC is thought to result from an arrest in compaction during early myocardial development, but a higher prevalence of LVNC in pregnant women and athletes suggests that LVNC can also occur as a physiological adaptation to pressure overload in healthy adults (34). The prevalence of LVNC remains unclear, as there is a wide variation in the reported prevalence, partly due to the absence of specific diagnostic criteria or “gold standard” and the use of different imaging techniques for diagnosis. In particular, in a meta-analysis of studies reporting LVNC prevalence in adults, the overall prevalence was estimated to 1.28% when diagnosed by echocardiography and 14.79% when based on cardiac magnetic resonance imaging (34). The clinical manifestation of LVNC is highly heterogeneous, ranging from asymptomatic to severe heart failure, arrhythmias, thromboembolism, LV dysfunction, and sudden cardiac death. Both sporadic and familial forms have been described, and LVNC can occur both in an isolated form and associated

with other cardiomyopathies. Up to 48% of cases are familial (35) and are most frequently caused by mutations in sarcomeric proteins but can also be caused by mutations in nuclear envelope, cytoskeletal, and mitochondrial proteins, transcription factors, and ion channels (32, 35). The inheritance of LVNC is mostly autosomal dominant or X-linked recessive, although autosomal recessive and mitochondrial inheritance also occur.

ACM is a progressive disease defined by cardiomyocyte death and fibrofatty replacement of the myocardium, leading to increased susceptibility to ventricular arrhythmias and sudden cardiac death (reviewed in Refs. 36–38). Furthermore, advanced stages of ACM are characterized by systolic dysfunction, biventricular dilation, and heart failure. ACM principally affects the right ventricle (RV), but cases with biventricular or principal LV involvement have become increasingly reported (36, 39, 40). For that reason, the disease was renamed from arrhythmogenic right ventricular cardiomyopathy (ARVC) or dysplasia (ARVD) to the broader term ACM in a consensus statement from the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA) (41). ACM affects ~1 in 1,000–5,000 but is likely underdiagnosed, as the initial manifestation may be sudden cardiac death. In particular, ACM is a leading cause of sudden death in athletes and young adults and has been estimated to be responsible for 10% of cardiovascular deaths in people under 65 yr of age (42, 43). Genetic mutations are responsible for up to 60% of cases and have been identified in 16 genes, mostly with autosomal dominant inheritance with variable penetrance (37), although autosomal recessive inheritance has also been reported. Mutations in genes encoding desmosomal proteins account for ~80% of cases with confirmed pathogenic mutations, while causative mutations have less frequently been identified in genes encoding sarcomeric, ICD, SR, and nuclear envelope proteins or growth factors. A common molecular mechanism underlying ACM is thought to be ICD remodeling due to mutations in desmosomal proteins, leading to disruption of cardiomyocyte adhesion and consequent apoptosis or necrotic cardiomyocyte death, resulting in cardiomyocyte loss and fibrofatty replacement (37, 38).

The major focus of the laboratory of Dr. Ju Chen, the senior author of this article, in the last two and half decades, has been to provide insights into the molecular basis of cardiomyopathy by using genetically engineered mouse models, physiological measurements, and a range of molecular and cell biological techniques. The present review is based on his 2021 Robert M. Berne Distinguished Lectureship of the American Physiological Society (APS) Cardiovascular Section. The lecture summarized the advancements in the understanding of the molecular mechanisms underlying human cardiomyopathies obtained in his laboratory. Thus, although this review aims to provide a general summary of the current understanding of the molecular basis of cardiomyopathy, it mainly focuses on the cardiomyopathy-associated proteins that have been studied in his laboratory (listed in Table 1).

THE SARCOMERE

The sarcomere is the smallest contractile unit of striated muscle in which interdigitating thick myosin filaments and

Table 1. Association of selected cardiac genes with human cardiomyopathies

Gene	Protein Name	Location	Type of Cardiomyopathy	Other Diseases	References
<i>LDB3</i>	Cypher/ZASP/LIM domain binding 3	Z-line	DCM, HCM, LVNC, ACM	MFM	(58–60, 62–63)
<i>CSRP3</i>	Muscle LIM protein (MLP)/cysteine and glycine-rich protein 3 (CSRP3)	Z-line, M-line, ICD, costamere, sarcolemma, nucleus	DCM, HCM		(95–103)
<i>NEBL</i>	Nebulette	Z-line	DCM, EFE, HCM, LVNC		(138–140)
<i>MYPN</i>	Myopalladin	Z-line, I-band, nucleus	DCM, HCM, RCM	NEM, CM, CAPM	(157–160)
<i>FHL1</i>	Four and a half LIM protein 1 (FHL1)	I-band, M-line	HCM, ACM, DCM	EDMD, RBM, SPM, XMPMA	(177–186)
<i>FHL2</i>	Four and a half LIM protein 2 (FHL2)	I-band, M-line	HCM, DCM		(194, 195)
<i>ANKRD1</i>	Cardiac ankyrin repeat protein (CARP)	I-band, nucleus	DCM, HCM		(212–214)
<i>MYL2</i>	Myosin light chain 2 (MYL2)/ventricular myosin light chain 2 (MLC-2v)	A-band	HCM, DCM	MFM	(265–285)
<i>OBSCN</i>	Obscurin	M-line, Z-line, A/I junction, costamere, ICD, SR	HCM, DCM, LVNC, ACM		(328–332)
<i>JUP</i>	Junction plakoglobin/ γ -catenin	ICD	ACM	NXD	(373–388)
<i>CXADR</i>	Coxsackievirus and adenovirus receptor (CAR)	ICD	Ischemia-induced ventricular fibrillation, elevated blood pressure (SNPs)		(414–416)
<i>TJP1</i>	Zonula occludens-1 (ZO-1)	ICD	ACM		(433)
<i>VCL</i>	Vinculin, metavinculin	Costamere, ICD	DCM, HCM		(485–489)
<i>ILK</i>	Integrin-linked kinase (ILK)	Costamere	ACM		(483, 533–535)
<i>FLNC</i>	Filamin C	Costamere, Z-line, ICD	HCM, RCM, DCM, ACM.	MPD, MFM	(482, 572–586)
<i>JPH2</i>	Junctophilin-2	Junctional SR	HCM, DCM		(627–631)
<i>NEXN</i>	Nexilin	Junctional SR	HCM, DCM		(654–656)
<i>SYNE1/SYNE2</i>	Nesprin-1/nesprin-2	Nuclear envelope	DCM	EDMD, SCARSCAR, AMC	(680, 682–689)
<i>TMEM43</i>	Luma	INM	ACM	EDMD	(715–722)
<i>TBX20</i>	T-box transcription factor 20 (TBX20)	Nucleus	Congenital heart disease, DCM, LVNC		(730–734)
<i>PRDM16</i>	PR domain-containing protein 16 (PRDM16)	Nucleus	DCM, LVNC		(755–761)
<i>BAG3</i>	BCL2-associated athanogene 3 (BAG3)	Cytosol, Z-line	DCM, RCM, HCM	MFM	(572, 807–819)
<i>EEF1A2</i>	Eukaryotic translation elongation factor 1 alpha 2 (eEF1A2)	Cytosol	DCM	DEE, MRD	(804–806)

ACM, arrhythmogenic cardiomyopathy; AMC, arthrogryposis multiplex congenita spinocerebellar; CAPM, cap myopathy; CM, congenital myopathy; DCM, dilated cardiomyopathy; DEE, developmental and epileptic encephalopathy; EDMD, Emery–Dreifuss muscular dystrophy; EFE, endocardial fibroelastosis; HCM, hypertrophic cardiomyopathy; ICD, intercalated disk; INM, inner nuclear membrane; LVNC, left ventricular noncompaction; MFM, myofibrillar myopathy; MPD, distal myopathy; MRD, mental retardation, autosomal dominant; NEM, nemaline myopathy; NXD, Naxos disease; RBM, reducing body myopathy; RCM, restrictive cardiomyopathy; SCAR, spinocerebellar ataxia, autosomal recessive; SNP, single-nucleotide polymorphism; SPM, scapuloperoneal myopathy; XMPMA, X-linked myopathy with postural muscle atrophy.

thin actin filaments slide past each other during muscle contraction and relaxation (3, 4). A third filament is formed by titin, the largest known vertebrate protein (3–3.7 MDa), which extends over 1 μ m from the Z-line to the M-line. Titin acts as a molecular spring responsible for the passive stiffness of striated muscle through the mechanical properties of its multiple domains (44, 45). Furthermore, it is important for a variety of processes, including sarcomere assembly and organization, force transmission, and mechanosensing (reviewed in Refs. 46, 47). Mutations in sarcomeric proteins have been associated with a wide range of congenital cardiomyopathies, including HCM, DCM, RCM, and LVNC (48). Below the different parts of the sarcomere are described and proteins associated with congenital cardiomyopathies are discussed.

Z-Line

The Z-line is a highly organized multiprotein complex at the boundary between sarcomeres in which actin and titin filaments from adjacent sarcomeres are anchored and cross-linked by α -actinin. α -Actinin plays a central role in connecting proteins at the Z-line and also interacts with a wealth of other Z-line proteins, including CapZ, nebullette, PDZ-LIM proteins, and members of the palladin/myopalladin/myotilin family (reviewed in Refs. 49–52). Z-lines are laterally aligned through a link to the intermediate filament desmin (53, 54) and connect myofibrils to the sarcolemma and extracellular matrix via the costamere (reviewed in Refs. 55, 56). Thus, in addition to a structural role in providing

structural stability, the Z-line is essential for efficient force production and transmission. Furthermore, the Z-line plays a key role in mechanosensing and transduction of biomechanical signals. Consequently, mutations in a large number of Z-line and Z-line-associated proteins have been linked to cardiomyopathies (49–51).

Genes encoding Z-line proteins associated with DCM include *LDB3* (cypher/ZASP), *CSRP3* (muscle LIM protein, MLP), *TCAP* (titin cap/telethonin), *ACTN2* (α -actinin 2), *MYPN* (myopalladin), *NEBL* (nebullette), and *PDLIM3* (PDZ and LIM domain 3/actinin-associated lim protein, ALP) (reviewed in Ref. 49). Furthermore, many Z-line proteins have been linked to HCM, including *MYOZ2* (myozenin-2/calsarcin-1), *LDB3*, *CSRP3*, *TCAP*, *ACTN2*, and *MYPN*. *MYPN* mutations have also been associated with RCM, *LDB3* and *ACTN2* mutations with LVNC, and *LDB3* mutations with ACM (listed in Ref. 49). Mutations located within the NH₂-terminal Z-line portion of titin have also been linked to DCM and in rare cases HCM and LVNC (46, 57). A major focus of our laboratory has been to dissect the role of Z-line proteins in cardiac function and disease, including cypher/ZASP, MLP, nebullette, and myopalladin, which are reviewed in detail below.

Cypher/ZASP.

Heterozygous missense mutations in the *LIM domain binding 3* (*LDB3*) gene, encoding cypher/ZASP, are causative for various human cardiomyopathies, including DCM (58, 59), HCM (60–62), LVNC (59, 62, 63), and ACM (63). In addition, *LDB3* mutations can cause skeletal muscle myopathies, referred to as zaspomyopathies (64–67). Cypher was first identified from mouse (68), whereas the human ortholog of cypher, Z-band alternatively spliced PDZ-motif protein (ZASP), was independently identified (69). In addition, cypher was independently cloned from mouse as Oracle (70). Cypher/ZASP is a striated muscle-specific PDZ-LIM protein located in the Z-line of the sarcomere. In mouse, six cypher isoforms have been identified, comprising four long isoforms and two short isoforms. All isoforms share an NH₂-terminal PDZ domain, whereas only long isoforms contain three COOH-terminal LIM domains (68, 71). Additionally, cypher contains a ZASP-like motif (ZM), also present in the other PDZ-LIM proteins ALP and CLP36, encoded by either exon 4 or exon 5–7, responsible for its cardiac or skeletal muscle specific expression, respectively (71, 72). One short and two long isoforms are specifically expressed in heart and skeletal muscle, respectively. In human, eight ZASP isoforms have been identified, which are similar in structure to murine cypher isoforms, with some minor differences (59, 69, 72, 73). However, as opposed to mouse, human isoforms containing exon 4 are expressed in both heart and skeletal muscle and isoforms containing exon 6 are highly expressed in both cardiac and skeletal muscle (73). This is important, as mutations both in exons 4 and 6 have been linked to cardiomyopathies (59).

Within the Z-line, cypher/ZASP directly interacts with α -actinin (68, 69, 74), myozenin-2/calsarcin-1/FATZ (75–77), and myotilin (76, 77) through its PDZ domain. Furthermore, the cypher/ZASP PDZ domain binds to ANKRD2 (73), tumor protein P53 (TP53) (73), and the L-type calcium channel (LTCC) (78). Long cypher/ZASP isoforms bind to protein kinase C (PKC) through their LIM domains, and cypher/ZASP

itself is a phosphorylation target of PKC (68, 79). Importantly, cypher/ZASP binds to the regulatory subunit RII α of protein kinase A (PKA), making it a PKA anchoring protein (AKAP) (78). In particular, cypher/ZASP is itself a target of PKA and was found to promote PKA-mediated phosphorylation of the LTCC, regulating channel activity (78, 80). Furthermore, cypher/ZASP interacts with glycolytic enzyme phosphoglucosyltransferase 1 (PGM1) (81) as well as binds to and inhibits the Ser/Thr phosphatase calcineurin, which also targets the LTCC (78). Thus, cypher/ZASP is important for striated muscle structure and signaling, and its requirement for normal cardiac function has been confirmed in studies of various mouse models as described below.

Conventional knockout (KO) of cypher in mouse resulted in postnatal death within 1–5 days after birth, associated with reduced milk intake, muscle weakness, cardiac dilation, and severely disorganized Z-lines (74). In addition, double KO of cypher and the PDZ-LIM protein Enigma homolog protein (ENH), both of which are dispensable for cardiac development, resulted in embryonic lethality by embryonic day 11.5 (E11.5), associated with cardiac dilation and Z-line disorganization, demonstrating that the two proteins play redundant roles in maintaining normal cardiac function and structure during embryonic development (82). Cardiomyocyte-specific deletion of cypher both postnatally and in adult heart resulted in severe DCM and premature death, accompanied by disorganization of the Z-line and altered cardiac signaling (77). Furthermore, although deletion of short cypher isoforms in mouse did not cause any detectable phenotypic abnormalities, ablation of long cypher isoforms resulted in partial neonatal lethality, with surviving mice showing growth retardation and late-onset DCM associated with Z-line abnormalities, cardiac fibrosis, calcification, and altered cardiac signaling (83). Moreover, cypher long isoform-specific KO mice showed an exaggerated pathological response to mechanical pressure overload or chronic β -adrenergic stress, characterized by cardiac dilation and cardiac systolic dysfunction (83). A recent in vitro study also suggested that cypher deficiency induces apoptosis (84), consistent with the finding that cypher acts as a negative regulator of the proapoptotic protein TP53 (73). To study the effect of the ZASP4 p.S196L mutation located in the cardiac-specific exon 4 and associated with human DCM with or without LVNC (59, 61, 62), Li et al. (85) generated transgenic (Tg) mice with cardiomyocyte-specific overexpression of wild-type (WT) or mutant ZASP isoform 4 (the longest cardiac-specific isoform). ZASP-S196L Tg mice developed late-onset DCM associated with mild fibrosis and ultrastructural abnormalities in the Z-line and sarcomere, recapitulating the clinical phenotype, whereas ZASP-WT Tg mice showed no detectable changes. This was preceded by electrocardiogram (ECG) abnormalities and altered L-type Ca²⁺ and Na⁺ currents, suggesting that cardiac conduction defects and atrioventricular block precede the development of DCM. Similarly, in cellular studies the ZASP1 p.D117N mutation was shown to cause loss of function of the Na_v1.5 cardiac voltage-gated sodium channel, predicted to cause cardiac conduction defects (86). Other in vitro studies showed that the ZASP p.D626N mutation located in the third LIM domain increases the binding affinity to PKC (58), whereas mutations located in exons 4 (ZASP p.S189L and ZASP p.T206I) and 10 (ZASP p.I345M) reduce the binding affinity to PGM1 (81).

Also, the PDZ-LIM proteins enigma homolog protein (ENH/PDLIM5) and actinin-associated LIM protein (ALP/PDLIM3) (87) may be involved in cardiac disease, as polymorphisms in the corresponding *PDLIM5* and *PDLIM3* genes have been associated with an increased risk of idiopathic DCM (88, 89) and *PDLIM5* mutations have been proposed as possible disease modifiers (90). Consistent with their possible role in cardiac pathology, global and cardiomyocyte-specific deletion of ENH in mouse resulted in DCM (91, 92), whereas ALP KO mice showed right ventricular chamber dilation and dysfunction (93, 94).

CSRFP3/muscle LIM protein.

Heterozygous missense and nonsense mutations in the *cysteine and glycine-rich protein 3* (*CSRFP3*) gene, encoding muscle LIM protein (MLP), have been associated with DCM (95–97) and HCM (98–103). Furthermore, reduced MLP protein levels have been reported in patients with DCM or ischemic cardiomyopathy (104). MLP is a 21-kDa striated muscle-specific protein present in the Z-line, M-line, ICD, costamere, plasma membrane, and nucleus (reviewed in Ref. 105). It contains two LIM domains and belongs to the LIM-only domain family (106). MLP can bind and bundle filamentous actin (F-actin) (107) and within the Z-line it binds to telethonin/T-cap (97, 108), α -actinin (109), cofilin-2 (110), calcineurin (111), and HDAC4 (112) as well as itself and the MLP-b isoform, containing the NH₂-terminal half LIM domain followed by 22 amino acids (113). At the ICD, MLP interacts with nebulin-related anchoring protein (NRAP) (114), whereas in the costamere it binds to zyxin (109), integrin-linked kinase (ILK) (115), and β 1-spectrin (116). Furthermore, MLP has been shown to contain a nuclear localization signal (NLS) and shuttle between the cytoplasm and the nucleus (117), where it interacts with MyoD, myogenin, and myogenic regulatory factor 4 (MRF4) (118). Within the nucleus, MLP is found in its monomeric form, whereas in the cytoskeleton and membrane it is present in its polymeric form (119). Accumulation of monomeric MLP in the nucleus and a decline in MLP oligomerization were reported in human failing hearts, suggesting that reduced nonnuclear MLP may contribute to heart failure (119).

Ablation of MLP in mouse resulted in death of 45–65% of homozygous KO mice within the first 2 wk after birth, whereas the rest survived to adulthood and developed eccentric hypertrophy, characterized by LV dilation, wall thinning, and systolic dysfunction (120). MLP KO mice exhibited interstitial fibrosis and severe disruption of myofibrillar organization (120), including misalignment of Z-lines (120, 121), altered costamere organization (114), abnormal ICD morphology and composition (114, 122), as well as mitochondrial disorganization and loss (123). Furthermore, before the development of DCM, MLP KO mice showed abnormal intracellular calcium handling (112, 121, 124, 125), alterations in passive myocardial properties (126, 127), and defective passive stretch sensing (97). Similarly, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) deficient for MLP showed typical features of HCM, such as increased cardiomyocyte size, multinucleation, and disorganized sarcomere structure, which progressed to mitochondrial dysfunction, increased reactive oxygen species (ROS) production, and impaired calcium handling, recapitulating heart failure. Importantly, restoration of

defective calcium handling with an L-type calcium channel blocker prevented the development of HCM and heart failure (128). While KO of MLP has detrimental effects, cardiac-specific overexpression of MLP in mouse did not affect cardiac morphology and function either under basal conditions or in response to mechanical pressure overload of chronic angiotensin II (ANG II) stimulation (129).

MLP KO mice have been used as a model of DCM in numerous studies aimed at reversing the cardiac pathological phenotype to provide insights into the molecular mechanisms leading to DCM and identify novel therapeutic targets. In particular, ablation of the SR calcium ATPase (SERCA2A) inhibitor phospholamban in MLP KO mice completely rescued the morphological, ultrastructural, molecular, and functional abnormalities of MLP KO mice and prevented the development of DCM (130), suggesting that defective SR calcium cycling plays a critical role in the progression toward heart failure in the MLP model. Transgenic overexpression of a peptide inhibitor of β -adrenergic receptor (β -AR) kinase 1 (β ARK1) also prevented the development of DCM and reversed the β -AR desensitization observed in MLP KO mice, whereas overexpression of β_2 -AR did not improve the phenotype (131). In a later study, β_2 -AR ablation was found to rescue the DCM phenotype of MLP KO mice, whereas β_1 -AR ablation worsened the phenotype (132). Notably, β_2 -AR ablation improved myocyte shortening and reversed pathological alterations in calcium handling (132). As inhibition of the calcium/calmodulin-dependent protein phosphatase calcineurin has been shown to have beneficial effects on cardiac hypertrophy, the effect of calcineurin inhibition in the MLP KO model of DCM was tested by generation of double-KO mice for the calcineurin A β catalytic subunit (111). This resulted in reduced cardiac function and enhanced apoptosis, cardiomyocyte death, and fibrosis, leading to early lethality. In contrast, modest overexpression of activated calcineurin was sufficient to improve systolic function and fibrosis (111). Also, ablation of the ANG II type 1a (AT1a) receptor in MLP KO mice was found to reduce ventricular dilation and improve systolic function but not the impaired calcium handling (133). More recently, ablation of cardiac ankyrin repeat protein (CARP/ANKRD1) or ankyrin repeat domain 2 (ANKRD2) in MLP KO mice, both upregulated in MLP KO mice, was found to rescue the DCM phenotype through a novel mechanism (134). More specifically, MLP was found to directly inhibit protein kinase C α (PKC α), and its ablation results in upregulation of CARP and Ankrd2, which accumulate at the ICD, leading to the formation of a multiprotein complex composed of CARP/ANKRD2, PKC α , and phospholipase-C β 1 (PLC β 1), resulting in detrimental chronic PKC α activation and consequent maladaptive remodeling and DCM. Ablation of CARP or ANKRD2 prevented the formation of the maladaptive signaling complex at the ICD, thus averting the development of DCM in MLP KO mice. Consistent with this mechanism, PKC α deletion or ablation reduced ventricular dilation and restored cardiac function in MLP KO mice (135, 136). It is possible that the other successful strategies for rescuing the DCM phenotype in MLP KO mice may also have affected maladaptive PKC α activation through other pathways.

The MLP p.W4R polymorphism, which has a frequency of ~0.0022 in the Genome Aggregation Database (gnomAD) exomes, has been associated with both DCM and HCM (96–99, 102, 108), suggesting that the outcome may be affected by

the effect of modifier genes, epigenetic factors, different environmental conditions, and/or incomplete penetrance (108). In biochemical studies, the variant was found to reduce the binding of MLP to telethonin, resulting in mislocalization of telethonin from the Z-line in a patient carrying the variant (97, 108). Knockin (KI) mice harboring the MLP p.W4R variant developed late-onset HCM as evidenced by increased LV wall and septal thickness, heart weight-to-body weight ratio, and fractional shortening as well as myofibrillar disarray and interstitial fibrosis (108). MLP was significantly downregulated in both heterozygous and homozygous MLP-W4R KI mice, and although MLP was less abundant in the Z-line, it was increased in the nucleus (108). Other MLP mutations have been studied in *in vitro* studies. The MLP p.K69L mutation, identified in a patient with DCM and endocardial fibroelastosis and located within the predicted NLS adjacent to the LIM1 domain, was found to affect the subcellular localization of MLP and abrogate the binding to α -actinin (96). Similarly, the MLP p.C58G HCM mutation, located in the LIM1 domain, reduced the binding affinity to both α -actinin and N-RAP (100, 137). In subsequent studies, the MLP p.C58G mutant was found to show reduced stability, suggesting that reduced levels of functional MLP may contribute to the development of cardiomyopathy. This is consistent with the finding that heterozygous MLP KO mice, which have reduced MLP levels, develop heart failure in response to myocardial infarction (111).

Nebulette.

Heterozygous missense mutations in the *NEBL* gene, encoding nebulette, have been associated with DCM with or without endocardial fibroelastosis, characterized by a thickening of the ventricular endocardium due to an increase in the amount of supporting fibrous and elastic tissue (138). Furthermore, rare heterozygous *NEBL* missense mutations have been identified in HCM and LVNC patients, without mutations in other known cardiomyopathy genes, suggesting that *NEBL* mutations may also be associated with HCM and LVNC (139). In addition, a *NEBL* polymorphism has been linked to idiopathic DCM in the Japanese population (140). Nebulette is a 107-kDa cardiac-specific protein located in the Z-line of the sarcomere. Like its larger skeletal muscle homolog nebulin, it is highly modular in structure, containing an NH₂-terminal glutamine-rich region, 23 35-residue nebulin-like repeats, a serine-rich linker region, and a COOH-terminal Src homology-3 (SH3) domain (141, 142). The nebulette SH3 domain binds to proline-rich regions in myopalladin (143), palladin (143), α -actinin (144), titin (145–147), zyxin (148), N-WASP/WASL (149), and XIRP1/2 (150). In addition, the NH₂-terminal acidic region of nebulette binds to filamin C (151), and the nebulin-like repeats bind to actin (144), α -actinin (144), troponin T (144), tropomyosin (144), and desmin (53). In particular, nebulette was found to be downregulated and show altered organization in desmin KO mice, and a role of nebulette in regulating the interaction between actin and desmin intermediate filaments has been proposed (53). Furthermore, nebulette expression studies in cardiomyocytes suggested a role of nebulette in myofibrillar organization and stabilization of the thin filament (152, 153).

Surprisingly, nebulette KO mice showed normal cardiac morphology and function both under basal conditions and in response to biomechanical stress despite upregulation of

cardiac pathological markers and progressive Z-line widening (154). In contrast, a mouse model in which exon 3 has been deleted, resulting in ablation of the first three nebulin-like repeats, developed diastolic dysfunction at 6 mo of age (155). Furthermore, nebulette exon 3-deleted mice showed exercise intolerance and developed eccentric hypertrophy and LV chamber dilation after chronic exercise, associated with disruption of ICDs and mitochondria (155). Tg mice overexpressing four different DCM-related *NEBL* mutations (p.K60N, p.Q128R, p.G202R, and p.A592E) in cardiomyocytes have also been reported (138). The *NEBL* p.K60N and p.Q128R mutations caused embryonic lethality, whereas the *NEBL* p.G202R, and p.A592E mutations led to DCM and systolic dysfunction at 6 mo of age, preceded by altered mechanical behavior by magnetic resonance imaging (decreased torsion in *NEBL*-G202R Tg hearts and increased twist and untwisting rate in *NEBL*-A592E Tg hearts), altered calcium handling, changes in the expression and localization of sarcomeric proteins, and ultrastructural abnormalities (138, 156). In particular, mitochondrial abnormalities and increased I-band width were observed in both models, whereas t-tubular enlargement and desmosomal separation at the ICD was observed in *NEBL*-G202R Tg mice (138, 156). For more insights into the effect of *NEBL* mutations, KI mice mimicking specific human mutations are needed.

Myopalladin.

Heterozygous missense and nonsense mutations in the *MYPN* gene, encoding myopalladin, have been associated with DCM, HCM, and RCM (157–160). Furthermore, biallelic *MYPN* loss-of-function mutations have been linked to nemaline myopathy (161), cap myopathy (162), and congenital myopathy with hanging big toe (163). Myopalladin is a 145-kDa striated muscle-specific protein present in both the nucleus and the sarcomere, where it has a dual localization in the Z-line and I-band (143). Myopalladin contains five immunoglobulin (Ig) domains and a proline-rich region and belongs to the palladin/myopalladin/myotilin family of actin-associated Ig-containing proteins in the Z-line (143, 164, 165). Within the cardiac Z-line, myopalladin interacts with α -actinin (143), nebulette (143), titin (166), and PDZ-LIM family members, including cypher/ZASP, CLP36, ALP, and RIL (76). Furthermore, it binds to the stress-inducible transcriptional cofactor cardiac ankyrin repeat protein (CARP/ANKRD1), which, like myopalladin, is present in both the nucleus and the I-band, where it binds to the titin N2A region (143, 167). Like the other family members, myopalladin also binds and bundles F-actin, preventing actin depolymerization (168). In addition, it interacts with myocardin-associated transcription factors (MRTF-A and MRTF-B), which act as cofactors for serum response factor (SRF), controlling its activity in response to alterations in actin dynamics (168).

Myopalladin KO mice developed a mild form of DCM but showed a maladaptive response to mechanical pressure overload induced by transaortic constriction (TAC) as characterized by LV dilation and severely impaired systolic function, accompanied by fibrosis, increased fetal gene expression, altered calcium handling, and increased ICD fold amplitude (166). Furthermore, Tg mice with cardiomyocyte-specific overexpression of the *MYPN* p.Y20C polymorphism, which has been linked to

both DCM and HCM, have been reported (159). MYPN-Y20C Tg mice developed HCM, associated with overexpression of fetal genes and severely disrupted ICDs together with alterations in the expression and localization of interaction partners and ICD proteins (159). KI mice for the nonsense MYPN p.Q529X mutation associated with RCM in patients have also been described (159, 169). Whereas homozygous MYPN-Q529X KI mice showed no pathological cardiac phenotype, supposedly due to very low expression of the mutant protein, heterozygous MYPN-Q529 KI mice showed diastolic dysfunction with preserved systolic function, cardiac arrhythmias, and interstitial fibrosis, consistent with RCM (169). Furthermore, enlarged t-tubules and widened, more convoluted ICDs were observed together with alterations in myopalladin-interacting proteins and cardiac signaling pathways.

Thin Filament

Actin is the main component of the thin filaments of the sarcomere, which consist of two strands of polymerized globular actin monomers (G-actin) twisted around each other to form double helical filaments (F-actin) (reviewed in Refs. 170, 171). F-actin polymerization is accompanied by ATP hydrolysis, and actin filaments undergo continuous treadmilling by F-actin polymerization and depolymerization. The barbed end of actin is anchored at the Z-line and capped by CapZ, whereas its pointed end reaches the A-band, where it is capped by tropomodulin. Along the actin filament, actin binds to tropomyosin dimers, each spanning seven actin subunits and associated with one troponin complex comprised of the three subunits T, I, and C, named after their tropomyosin binding, inhibitory, and calcium binding activities during muscle contraction. In the absence of calcium, tropomyosin sterically hinders the actomyosin interaction. Upon release of calcium from the SR following excitation, calcium binds to troponin, causing tropomyosin to move over the surface of actin, exposing myosin-binding sites on the actin filament. Myosin heads of the thick filament can then bind to actin subunits and form myosin cross bridges, allowing for the production of force and muscle contraction (172).

Several genes encoding proteins associated with the thin filament have been linked to cardiomyopathies. Mutations in *TNNT2* (troponin T2), *TNNI3* (troponin I3), *TPM1* (tropomyosin 1), *TNNC1* (troponin C1), and *ACTC1* (α -cardiac actin) have been associated with HCM, DCM, and, less frequently, RCM (28, 173, 174). Furthermore, mutations in *ACTC1*, *TNNT2*, *TPM1*, and *TNNI3* have been linked to LVNC (31), and *ACTC1* mutations have been associated with atrial septal defects (175). Mutations in the I-band region of titin have also been associated with various forms of cardiomyopathy, such as DCM and, less frequently, ACM, HCM, and RCM (46, 176). Four and a half LIM proteins (FHLs) and cardiac ankyrin repeat proteins (CARPs) bind to the titin N2B and N2A regions in the I-band, respectively and have been associated with various forms of cardiomyopathies. These proteins have been extensively studied in our laboratory and are described in detail below.

Four and a half LIM proteins.

Mutations in the four and a half LIM proteins FHL1 and FHL2, also known as skeletal muscle LIM (SLIM) 1 and 3, have been

associated with human cardiomyopathies. In particular, a number of studies have associated mutations in *FHL1*, positioned on the X chromosome, with HCM (177–184). Furthermore, *FHL1* mutations have been linked to various myopathies with cardiac involvement, including Emery–Dreifuss muscular dystrophy (EDMD) with HCM (179) or ACM (185), X-linked recessive distal myopathy with HCM (181), and reducing body myopathy with DCM (186). In addition, FHL1 is up-regulated during hypertrophy in both human (187–190) and mouse (191–193). Missense mutations in *FHL2* have been associated with HCM (194) and DCM (195). Furthermore, reduced FHL2 transcript levels have been found in heart failure patients and during cardiac hypertrophy in both human (194) and mouse (187, 194). FHL proteins have a molecular mass of 32 kDa and contain four LIM domains and an NH₂-terminal half LIM domain. FHL1 is highly expressed in skeletal muscle and to a lesser extent in heart, whereas FHL2 is nearly exclusively expressed in heart (191). Both proteins are localized at the I-band and M-line of the sarcomere (196, 197) and interact with a wealth of proteins, including structural proteins, ion channels, receptors, and signaling proteins (reviewed in Refs. 198, 199). In particular, both proteins interact with the elastic titin N2B region in the I-band of the heart (193, 196), and FHL1 was shown to be part of a biomechanical stress sensor complex targeting MAPKs (Raf1, MEK2, ERK2) to the titin N2B region and function as a positive regulator of ERK-mediated signaling in response to hypertrophic stimuli (193).

FHL1-deficient mice exhibited normal cardiac morphology and function under basal conditions (193) but developed age-dependent skeletal muscle myopathy, associated with structural changes and decreased life span (200). In response to mechanical pressure overload induced by TAC, FHL1 KO mice showed a blunted hypertrophic response but preserved LV function, as characterized by reduced LV weight-to-body weight ratio, cardiomyocyte cross-sectional area, LV wall thickness, and fetal gene expression compared with WT mice (193). In particular, although WT mice showed reduced systolic function after TAC, the systolic function of FHL1 KO mice was comparable to that of sham-operated mice. Similarly, FHL1 ablation was sufficient to prevent pathological hypertrophy, LV dilation, and systolic dysfunction in Tg mice overexpressing constitutively active $G\alpha_q$ in cardiomyocytes (201), indicating that FHL1 plays an essential role in $G\alpha_q$ -mediated hypertrophic signaling (193). Consistent with a role of FHL1 in biomechanical stress sensing, FHL1 KO mice showed reduced ERK1/2 signaling following TAC, loss of stretch-induced hypertrophic signaling, and increased muscle compliance (193, 202). Surprisingly, ablation of FHL1 in a myosin heavy chain (MHC) p.R403Q KI mouse model of cardiac hypertrophy (203), showing upregulation of a long FHL1 isoform and downregulation of FHL2, resulted in the development of a more severe HCM phenotype (187). The authors hypothesized that the detrimental effect could be due to downregulation of FHL2, which is not altered in FHL1 KO mice and is a negative regulator of hypertrophy (204). KI mice for the FHL1 p.W122S mutation associated with X-linked scapuloperoneal myopathy were recently described (205, 206). In contrast to male mice, which developed late-onset, slowly progressive myopathy without cardiac involvement (207), female mice showed no skeletal myopathy but developed late-onset cardiac systolic dysfunction associated

with enlarged rectangular nuclei (205). Thus, consistent with the location of FHL1 on the X chromosome, FHL1 mutations can have sex-specific effects.

FHL2 KO mice were initially reported to show normal cardiac function both under basal conditions and in response to mechanical pressure overload by TAC (208). However, in subsequent studies, FHL2 KO mice were demonstrated to show worsened cardiac hypertrophy in response to chronic infusion of the β -adrenergic agonist isoproterenol (ISO) (204, 209). Consequently, FHL2 has been proposed to play a protective role in the heart by inhibiting β -adrenergic signaling and hypertrophy through inhibition of calcineurin (209) and ERK signaling (210), both promoting cardiac hypertrophy. A beneficial effect of FHL2 on the hypertrophic response was demonstrated in cardiomyocyte-specific Rho-associated coiled-coil containing kinase 2 (ROCK2) KO mice, which showed a blunted hypertrophic response to TAC or chronic ANG II infusion associated with upregulation of FHL2 and reduced ERK2 phosphorylation (211). Double heterozygous KO mice for ROCK2 and FHL2 showed a restored hypertrophic response to ANG II and ERK phosphorylation levels, demonstrating that the antihypertrophic response of ROCK2 KO mice is dependent on FHL2 (211). Thus, FHL1 promotes stress-induced cardiac pathological hypertrophy, whereas FHL2 plays a protective role by negatively regulating cardiac hypertrophy.

ANKRD1/cardiac ankyrin repeat protein.

Heterozygous missense mutations in *ANKRD1*, encoding cardiac ankyrin repeat protein (CARP), have been associated with DCM (212, 213) and HCM (214), with frequencies of ~2% and 0.8%, respectively. Furthermore, increased CARP levels have been reported in patients with various forms of cardiomyopathy, including DCM, HCM, ACM, and ischemic cardiomyopathy (215–219). CARP is a 36-kDa protein belonging to the muscle ankyrin repeat protein family of stress-inducible proteins, also including ankyrin repeat domain protein 2 (ANKRD2/ARPP) and diabetes-related ankyrin repeat protein (DARP/ANKRD23) (167). All family members contain a NLS, a coiled-coil region, four ankyrin repeats, and a PEST sequence, responsible for their rapid turnover by the ubiquitin-proteasome pathway (167, 220, 221). CARP is highly expressed in the early embryonic heart but downregulated in adult heart (222–224). However, it is highly induced in response to various stress conditions, such as biomechanical stress or stimulation with adrenergic agonists or ANG II, and is part of the fetal gene program that is induced during cardiac remodeling (120, 215, 223, 225–228). Like the other family members, CARP has a dual localization in the nucleus and the sarcomeric I-band, where it binds to the titin N2A region within the elastic I-band region of titin (167, 229, 230). In particular, CARP was found to affect PKA-dependent phosphorylation of the titin N2A region (229, 231, 232) and induce cross-linking of titin and actin myofilaments (233, 234), suggesting a role of CARP in increasing sarcomere stiffness and stability to preserve muscle mechanical performance under conditions of stress, possibly also influencing mechano- and phosphorylation-dependent signaling. A role of CARP in cardiac signaling was suggested by biochemical and cellular studies, where CARP was found to bind to YB-1 (224) and function as a nuclear transcriptional cofactor by

negatively regulating the expression of cardiac genes such as *Nppa*, *Myl2*, and *Tnnc1* (222–224, 235, 236). Furthermore, CARP is able to dimerize (237) and bind to myopalladin (143, 167), desmin (237), calsequestrin 2 (238), talin-1 (213), FHL2 (213), MuRF1 (239), MuRF2 (239), TP53 (240), nucleolin (241), PLC β 1 (242), and 14-3-3 proteins (236). In addition, CARP coprecipitates with GATA4 (243–245) and the p50 subunit of NF- κ B (246) as well as affects their activity, but it is unknown whether it directly binds to these proteins.

Surprisingly, CARP KO mice exhibit no basal phenotype and show a normal hypertrophic response to mechanical pressure overload induced by TAC (245, 247). However, they show complete attenuation of phenylephrine-induced cardiac hypertrophy (245). Furthermore, CARP ablation rescued the DCM phenotype of MLP KO mice by preventing PKC α accumulation at the ICD (see *CSRP3/muscle LIM protein*) (242). In-line with this, CARP was reported to enhance ANG II-induced cardiomyocyte hypertrophy and apoptosis in vitro, and myocardial injection of adenovirus expressing CARP after TAC led to increased cardiac hypertrophy and apoptosis, associated with exacerbated cardiac dysfunction, whereas CARP knockdown inhibited TAC-induced hypertrophy and apoptosis (248, 249). Similarly, the selective ANG II inhibitor olmesartan prevented ANG II-stimulated upregulation of CARP in vitro and likewise reduced CARP upregulation and cardiac hypertrophy in mice subjected to TAC (249). In contrast, Tg mice overexpressing CARP in cardiomyocytes were reported to exhibit no basal cardiac pathological phenotype and show attenuated TAC- and isoproterenol (ISO)-induced hypertrophy through inhibition of Erk1/2 and transforming growth factor (TGF)- β signaling (250). Thus, the role of CARP in cardiac hypertrophy remains controversial, although the discrepancy may be explained by the difference between permanent versus transient CARP overexpression. More recently, CARP Tg mice generated by another group were reported to be born with a sinus venosus defect as a result of impaired remodeling of the early embryonic heart (251). Consequently, adult CARP Tg mice showed diastolic dysfunction with preserved ejection fraction, which developed into heart failure at ~10 mo of age, associated with impaired relaxation, left atrial enlargement, sarcomeric disorganization and loss, mitochondrial damage, and accumulation of lipid droplets (251). The development of sinus venosus defect in CARP Tg mice is consistent with the identification of *ANKRD1* as a candidate gene for total anomalous pulmonary venous return (TAPVR), which has been associated with increased *ANKRD1* transcript levels (252). Because of the late development of gross cardiac abnormalities in CARP Tg mice, it is possible that the basal phenotype may have been missed in the previously published study (250).

As of yet no mouse models for specific *ANKRD1* mutations have been generated. However, various *ANKRD1* mutations associated with DCM and HCM have been studied in vitro. *ANKRD1* DCM mutations were found to have various effects dependent on their location, including reduced binding to talin-1 and/or FHL2, blunted *Mlc2v* repressor activity, decreased CARP-mediated inhibition of phenylephrine-induced cardiomyocyte hypertrophy, and altered stretch-induced signaling, whereas CARP localization was unaffected (212, 213). On the other hand, *ANKRD1* HCM mutations were reported to increase the binding affinity of CARP

for titin and MYPN (titin mutations at the CARP-binding site likewise increased binding affinity), cause nuclear translocation of CARP, and affect cardiac contractile parameters (214, 253). This is consistent with the general proposed mechanism, which seems to apply to many sarcomeric genes, that gain-of-function mutations result in HCM, whereas loss-of-function mutations cause reduced contractility and DCM (254).

Thick Filament

The bipolar thick filament is formed by myosin molecules positioned with their globular heads facing outward and their rod regions facing inward (reviewed in Refs, 255, 256). Myosin is a hexamer consisting of two myosin heavy chains, two essential light chains, and two regulatory light chains. The heavy chains are organized into three structural domains: the globular head domain containing actin and ATP binding sites, the lever arm domain containing essential and regulatory light chains, and the tail region containing an α -helical coiled-coil region required for dimerization to form rods. During muscle contraction the myosin heads attach to opposite oriented thin filaments and pull them toward the center of the sarcomere. Within the central region (C zone) of the A-band, myosin-binding protein-C (MyBP-C3) binds to myosin along the filament, while simultaneously interacting with titin and actin, forming 7–11 transverse stripes (257) (reviewed in Ref. 258). MyBP-C3 plays an important role in stabilizing the thick filament and modulating the actomyosin interaction.

Mutations in *MYBPC3* (cardiac myosin-binding protein C) are responsible for 40–50% of familial HCM cases and have also been associated with DCM, LVNC, and RCM (reviewed and summarized in Refs. 173, 256, 259). Mutations in *MYH7* (myosin heavy chain 7, also known as β -myosin heavy chain) account for ~40% of congenital HCM cases and have also been linked to DCM and LVNC. Also, mutations in *MYH6* (myosin heavy chain 6, also known as α -myosin heavy chain) have been associated with HCM, DCM, and atrial septal defects. Mutations in *MYL2* and *MYL3* (myosin light chain 2 and 3) are less frequent causes of HCM, and *MYL2* mutations have also in rare cases been associated with DCM (reviewed in Ref. 260). Furthermore, a truncating mutation in *MYBPHL* (myosin-binding protein-H like) was recently associated with DCM and arrhythmia (261). A high number of mutations in the A-band region of titin have been associated with DCM (listed in Refs. 46, 256), and titin-truncating mutations in the A- and M-line regions have been estimated to be responsible for 25–30% of familial cases of DCM (262, 263). Additionally, a small number of missense mutations have been linked to ACM (46, 264). *MYL2*, which has been studied in our laboratory, is reviewed in detail below.

Myosin light chain 2.

Heterozygous mutations in *MYL2*, encoding myosin light chain 2 (MYL2), also known as ventricular myosin light chain 2 (MLC2v), have been associated with HCM (265–281) and, more rarely, DCM (282, 283). Furthermore, a recessive *MYL2* mutation caused severe DCM, resulting in infantile death (284), whereas a homozygous frameshift variant, resulting in loss of function, caused HCM, resulting in death before 1 yr

of age (285). *MYL2* is a 19-kDa protein expressed in adult ventricular and slow-twitch skeletal muscle (286, 287). In the heart, myosin is composed of two heavy chains, two essential light chains in the NH₂-terminal half of the neck, and two regulatory light chains (MYL2) at the neck/tail junction (288).

Ablation of *MYL2* in mouse resulted in embryonic lethality by E12.5, associated with myofibrillar disorganization and sarcomere misalignment, cardiac dilation, and severe systolic dysfunction despite compensatory upregulation of the cardiac atrial myosin light chain 2 (MYL7/MLC2a) isoform, which is mainly expressed during embryonic development and normally present only in the atria of the adult heart (71, 287). Interestingly, heterozygous *MYL2* KO mice showed normal *MYL2* protein expression levels despite a 50% reduction at the mRNA level and consequently showed no pathological phenotype either under basal conditions or after TAC (289). The function of *MYL2* is regulated by phosphorylation by cardiac myosin light chain kinase 3 (MYLK3/cMLCK) at Ser15 in human and Ser14/Ser15 in mouse (reviewed in Refs. 290, 291). In particular, through analysis of a nonphosphorylatable *MYL2*-S14A/S15A KI mouse model and computational modeling (292), *MYL2* phosphorylation was found to regulate cross-bridge cycling kinetics and fine-tune the myofilament calcium sensitivity to force by increasing myosin lever arm stiffness and myosin head diffusion, thereby slowing down myosin cycling kinetics and prolonging the cross-bridge duty cycle. *MYL2*-S14A/S15A KI mice showed an early increase in the rate of cardiac muscle twitch relaxation and reduction in ventricular peak torsion preceding the development of a DCM phenotype and premature death (292). Furthermore, *MYL2*-S14A/S15A KI mice showed a maladaptive response to TAC-induced pressure overload, to which they showed an eccentric rather than concentric response, resulting in severe systolic dysfunction (292). *MYLK3* KO and hypomorphic mice, showing loss or reduction of *MYL2* phosphorylation, developed similar phenotypes (293, 294), whereas Tg mice overexpressing *MYLK3* specifically in cardiomyocytes showed an attenuated response to TAC (294). Similarly, Tg mice overexpressing myosin phosphatase 2 in cardiomyocytes, resulting in reduced *MYL2* phosphorylation, likewise developed a DCM phenotype (295). On the other hand, Tg mice with cardiomyocyte-specific overexpression of the *MYL2*-S14A/S15A mutant showed atrial defects, but no DCM phenotype, as well as a blunted contractile response to β -adrenergic stimulation (296, 297), likely due to the different strategies used and different levels of *MYL2* dephosphorylation obtained. Consistent with a role of *MYL2* dephosphorylation in DCM, *MYL2* dephosphorylation has been reported in human patients with DCM and heart failure (298–302), and reduced *MYL2* protein levels as a result of proteinase-mediated cleavage have been found in DCM patients (303). Furthermore, *MYL2* dephosphorylation has been reported in patients with a rare form of familial HCM carrying specific *MYL2* mutations (281, 304).

A number of Tg mouse-lines overexpressing specific *MYL2* disease mutants in cardiomyocytes have been extensively characterized. Tg mice overexpressing the human *MYL2* p.E22K mutant associated with HCM were reported to show mildly enlarged interventricular septa and papillary muscles (305), whereas Tg mice overexpressing the corresponding

mouse mutant did not show any sign of hypertrophy (306). Functional analyses of MYL2-E22K Tg mice showed increased calcium sensitivity of myofibrillar ATPase activity and force (305). The mutation had no effect on cross-bridge kinetics but caused a reduction in maximal force and ATPase in skinned fibers as well as decreased magnitude and duration of force and calcium transients in electrically stimulated muscle fibers (307, 308). On the other hand, prolonged calcium transients were reported in electrically stimulated muscle fibers from Tg mice expressing the human MYL2 p.N47K and p.R58Q HCM mutants (309, 310). In addition, MYL2-R58Q Tg mice showed a prolonged force transient, decreased cross-bridge kinetics, as well as increased calcium sensitivity of ATPase and steady-state force. MYL2-N47K and MYL2-R58Q Tg mice did not develop HCM, but MYL2-R58Q Tg mice showed reduced MYL2 phosphorylation and impaired relaxation and diastolic dysfunction (265, 274). In a subsequent study, adeno-associated virus 9 (AAV9)-mediated specific RNA silencing in MYL2-N47K Tg mice, reducing expression of the mutated allele, was shown to ameliorate the disease phenotype by reducing fetal gene expression and partially restore contraction, relaxation, and calcium kinetics (311). As in MYL2-R58Q Tg mice, Tg mice overexpressing the human MYL2 p.D166V HCM mutant showed decreased MYL2 phosphorylation, delayed muscle relaxation, reduced maximal myofibrillar ATPase and force, and increased calcium sensitivity of contractile force (312), which could be reversed by MYLK-induced phosphorylation of myofibrils (313). Constitutive MYL2 phosphorylation, obtained by generation of Tg mice expressing the MYL2 p.D166V mutant with constitutive phosphorylated Ser15 (MYL2-S15D-D166V), was subsequently shown to reverse the functional effects of the D166V mutation and prevent the development of HCM (314). Similarly, AAV9-mediated delivery of MYL2-S15D to MYL2-R58Q Tg mice improved cardiac performance, suggesting its therapeutic potential (315). A Tg mouse model overexpressing the human MYL2 p.K104E HCM mutant showed diastolic dysfunction before the development of late-onset HCM and fibrosis, associated with reduced MYL2 phosphorylation, reduced maximum tension, impaired muscle relaxation, and inefficient energy use (316). A subsequent gene expression profiling study on MYL2-R58Q, MYL2-D166V, and MYL2-K104E Tg mice showed distinct gene expression patterns, suggesting that the three HCM mutations lead to HCM through different mechanisms (317). A Tg mouse overexpressing the human MYL2 p.D94A mutant associated with DCM was also recently reported (318). MYL2-D94A Tg mice developed DCM, mimicking the clinical phenotype. This was associated with structural alterations in the myosin head affecting its interaction with the actin filament, resulting in aberrant cross-bridge cycling and reduced calcium sensitivity of force, ultimately leading to systolic dysfunction (318). The effects of the MYL2 p.R633H (319) and p.R58Q (320) HCM mutations have also been studied in patient-specific hiPSC-CMs. HiPSC-CMs carrying the MYL2 p.R58Q mutation, which has been extensively studied in Tg mice, showed cardiomyocyte hypertrophy, myofibrillar disarray, irregular beating, decreased calcium transients, and reduced LTCC peak current, recapitulating the human HCM phenotype (320). Similarly, hiPSC-CMs carrying the MYL2 p.R633H mutation showed increased cardiomyocyte size, upregulation of hypertrophic marker genes, arrhythmia

at the single-cell level, dysregulation of calcium cycling, and elevation of intracellular calcium, recapitulating common features of HCM (319). Importantly, pharmacological restoration of calcium homeostasis prevented the HCM phenotype, suggesting that patient-derived hiPSC-CMs may provide a useful system for testing of novel therapies. Overall, although the extensive characterization of Tg mice overexpressing human MYL2 mutants associated with disease has provided important insights into the disease mechanisms leading from MYL2 mutations to cardiomyopathy, the majority of the models do not recapitulate the human HCM phenotypes. In the future, the generation of KI models, which more closely mimic the human condition, would be helpful to better understand the disease mechanisms and provide more appropriate models for the testing of potential therapies.

M-Line

The M-line is located at the center of the A-band, where the rods of antiparallel myosin arrays and the COOH termini of titin from each half-sarcomere overlap (reviewed in Refs. 268, 321, 322). Furthermore, the M-line contains the three structurally related proteins, myomesin 1, myomesin 2/M-protein, and myomesin 3, that form antiparallel dimers interacting with myosin and titin, thereby cross-linking adjacent myosin and titin filaments (M bridges) to maintain thick filament alignment and stabilizing the sarcomere during contraction. The M-line also contains other proteins, such as muscle creatine kinase (CKM), obscurin, obscurin-like 1, MyBP-C1, FHLs, muscle-specific RING finger proteins (MURFs), ankyrins, and spectrins, implicating it in multiple cellular processes, including signal transduction, mechanosensing, metabolism, and protein turnover.

Patients homozygous or compound heterozygous for truncating mutations in the COOH-terminal M-line portion of titin have been reported to develop skeletal myopathy with DCM and/or other cardiac disorders, such as LVNC, septal defects, and cardiac arrhythmia, resulting in early cardiac death (323, 324), whereas heterozygous missense mutations in the COOH-terminal region of titin have been associated with various forms of skeletal myopathy with no cardiac involvement. Furthermore, a mutation in *MYOM1* (myomesin 1) has been associated with HCM (325), and a potential link of a *MYOM3* (myomesin 3)-truncating mutation to DCM was proposed (326). Additionally, increased levels of the embryonic heart myomesin (EH-myomesin) isoform were reported in myocardial biopsies from DCM patients (327). Mutations in *OBSCN* (obscurin) have been linked to various cardiomyopathies (summarized in Ref. 256), and the current knowledge on obscurin is summarized below.

Obscurin.

Missense and frameshift mutations in the *OBSCN* gene, encoding obscurin, have been associated with HCM (328, 329), DCM (330, 331), LVNC (331), and ACM (332). Furthermore, obscurin isoform switching was found in the LV of DCM patients (333), and increased obscurin transcript levels were reported in a canine model of tachycardia-induced DCM (334) as well as in mice subjected to mechanical pressure overload by TAC (335). Obscurin is a myosin light chain kinase belonging to a family

of three related proteins, including striated preferentially expressed gene (SPEG) and obscurin-like 1 (OBSL1). In striated muscle, several obscurin isoforms are expressed, including two large isoforms designated obscurin-A (720 kDa) and obscurin-B (870–970 kDa), which have different COOH termini. Obscurin-A and obscurin-B share ~70 Ig-like domains, 2–3 fibronectin III (FN3)-like domains, a calmodulin IQ-binding motif, a SRC homology 3 (SH3) domain, a rho-guanine nucleotide exchange factor (RhoGEF) domain, and a pleckstrin homology (PH) domain. However, whereas the COOH-terminal region of obscurin-A, the dominant isoform in the heart, comprises a nonmodular region and several potential phosphorylation sites, the COOH-terminal region of obscurin-B contains two Ser/Thr kinase-like domains, two additional Ig domains, and a FN3 domain (336–339). In addition, two shorter isoforms with predominant expression in the heart have been characterized, corresponding to the COOH-terminal region of obscurin-B and containing one [“single” obscurin-myosin light chain kinase (s-MLCK), 55–70 kDa] or two [“tandem” obscurin-myosin light chain kinase (t-MLCK), 120–150 kDa] kinase-like domains (336, 339, 340). Two small isoforms, obscurin-40 (40 kDa) and obscurin-80 (80 kDa), containing the PH domain and localized at the ICD, were also recently described and shown to be reduced in mouse models of pressure overload and myocardial infarction (341). Obscurin is present in different locations of the cell, including the M-line, Z-line, and A/I junction of the sarcomere as well as the costamere, ICD, and SR (reviewed in Ref. 342). At the M-line, obscurin interacts with titin (MI0) (343), myomesin (343), RhoA (344), and slow skeletal myosin-binding protein C (MyBPC1) (345), whereas at the Z-line it interacts with titin (Z9-Z10 and the novex-3 titin isoform) (338, 346) and RAN binding protein 9 (RanBP9) (347). Furthermore, the obscurin-A isoform binds to small ankyrins (ANK1, ANK2) at the M-line and SR (348–351), whereas the kinase domains of the obscurin-B isoform can bind to Na⁺-K⁺-ATPase (ATPIA1) and phosphorylate N-cadherin at the ICD (352). Additionally, the PH domain present in the two recently identified obscurin-40 and obscurin-80 isoforms binds to phosphatidylinositol phosphates [phosphatidylinositol 4,5-bisphosphate (PIP₂)] (341). Although the exact function of obscurin is still not well understood, it is thought to be involved in myofibril assembly and provide structural stability by connecting sarcomeric complexes to the SR and sarcolemma, potentially also implicating it in mechanosensing and signaling (reviewed in Refs. 321, 342, 353).

Obscurin KO mice showed normal sarcomeric organization and cardiac and skeletal muscle function but exhibited altered longitudinal SR architecture in skeletal muscle as well as disrupted sANK1.5 expression and localization in both cardiac and skeletal muscle (354, 355). Furthermore, obscurin KO mice showed reduced exercise performance depending on age and exercise intensity, which was associated with ultrastructural abnormalities in the diaphragm (349, 356). The mild phenotype of obscurin KO mice was thought to be due to the presence of nontargeted COOH-terminal isoforms and/or compensation by its homolog obscurin-like 1. Characterization of double skeletal muscle KO mice for obscurin and obscurin-like 1 mice confirmed the redundant role of the two proteins in sarcolemmal integrity, SR organization, and muscle metabolism (357), although it

remains to be determined whether they play redundant roles also in the heart. More recently, homozygous KI mice for the OBSCN p.R4344Q variant in obscurin Ig58 associated with HCM were described (358). OBSCN-R4344Q KI mice showed no obvious morphological and functional abnormalities in the heart, but at 1 yr of age OBSCN-R4344Q KI mice displayed increased SR calcium content, faster contraction and relaxation kinetics, and spontaneous ventricular arrhythmia, associated with upregulation and overactivation of SERCA2. Furthermore, OBSCN-R4344Q KI mice showed a maladaptive response to TAC-induced pressure overload, after which they developed DCM and interstitial fibrosis (358). The phenotype was thought to be attributed to increased affinity of obscurin Ig58 for its newly identified binding partner phospholamban (SERCA2 inhibitor), which was found to be reduced in OBSCN-R4344Q KI hearts. This was subsequently challenged by Fukuzawa et al. (359), who confirmed the binding in vitro but found no interaction or colocalization between the two proteins in cotransfected cells and thus suggested that the interaction is an in vitro artifact. They also did not confirm the previously reported impaired binding of the OBSCN p.R4344W mutant to titin Z9-Z10 (328). Consistent with these considerations, the pathogenicity of the OBSCN p.R4344Q variant has been questioned, as it has been identified in up to 15% of Black Americans and found to have an overall allele frequency of 0.01165 (330, 360). Nevertheless, the importance of the obscurin Ig58/59 region was recently demonstrated in a mutant mouse model expressing obscurin lacking Ig58/59 (OBSCN-ΔIg58/59) (361). Young OBSCN-ΔIg58/59 mice exhibited no basal phenotype but showed cardiac arrhythmia after acute β-adrenergic stimulation. At 6 mo of age, OBSCN-ΔIg58/59 mice showed LV hypertrophy, which developed into DCM with atrial enlargement, systolic dysfunction, and severe arrhythmia by 12 mo of age. This was associated with abnormal calcium handling and altered expression and phosphorylation of SR proteins, suggesting a role of the obscurin Ig58/59 region in calcium handling. The stronger pathological phenotype compared with obscurin KO mice suggests that the Ig58/59 deletion has a dominant negative effect, while proteins with redundant functions, such as obscurin-like 1, may compensate for the complete absence of obscurin. A recently identified frameshift mutation in *OBSCN* (L5218fs) associated with ACM was recently studied in patient-derived hiPSC-CMs and found to cause lipid accumulation, increased pleomorphism, Z-line irregularities, increased L-type calcium currents, mislocalization and reduced expression of ANK1.5, downregulation of desmosomal genes, and upregulation of N-cadherin and adipogenesis pathway-related genes (332). This is consistent with fibrofatty replacement in the myocardium of ACM patients and may provide a molecular basis for the development of arrhythmia in ACM patients carrying the frameshift mutation.

■ INTERCALATED DISK

The intercalated disk (ICD) is a highly organized structure connecting adjacent cardiomyocytes to each other, essential for synchronized contraction and the maintenance of structural integrity (reviewed in Refs. 5–7). The ICD is composed of three major junctional complexes: desmosomes, which link the cell membrane to the intermediate filament desmin;

fascia adherens junctions, which connect the cell membrane to the actin filament, facilitating the transmission of contractile force between cardiomyocytes; and gap junctions, which allow for the passage of ions and small molecules between neighboring cardiomyocytes, enabling electrical and metabolic coupling between cells. Rather than functioning as distinct units, the three junctions collaborate through interaction between their components in mixed junctions termed “area composita,” facilitating mechanical and electrical coupling as well as providing structural stability.

Mutations in many components of the ICD have been associated with human disease. Mutations in genes encoding desmosomal proteins are responsible for 85–90% of familial cases of ACM, including *PKP2* (plakophilin-2), *DSP* (desmoplakin), *DSG2* (desmoglein-2), *DSC2* (desmocollin-2), and *JUP* (plakoglobin/ γ -catenin) (reviewed in Ref. 37). Furthermore, *DSP* mutations have been linked to DCM with woolly hair and keratoderma (362), *PKP2* mutations have been associated with catecholaminergic polymorphic ventricular tachycardia (363), and a polymorphism in *DSG2* has been associated with increased risk of DCM (364). Also mutations in genes encoding other ICD-associated proteins have been linked to ACM, including *CTNNA3* (α -T-catenin), *CDH2* (N-cadherin), *DES* (desmin), *TJPI* (tight junction protein-1/zonula occludens-1), *SCN5A* (voltage-gated sodium channel alpha subunit 5), and *TMEM43* (transmembrane protein 43) (reviewed in Ref. 37). Furthermore, *DES* mutations have been associated with DCM and, less frequently, HCM, RCM, and LVNC (reviewed in Ref. 365). In animal models, genetic modification of the fascia adherens junction proteins N-cadherin (366–368) and α -E-catenin (366, 369) resulted in DCM, whereas ablation of xin actin binding repeat containing 1 (XIRP1) caused HCM (370). However, as of yet, mutations in these proteins have not been associated with human DCM or HCM. No direct link between mutations in gap junction proteins and human disease has been established, although a potential link between mutations in the gap junction protein connexin 43 (*GLA1*) and sudden cardiac death has been reported (371, 372). The ICD proteins plakoglobin, coxsackievirus and adenovirus receptor (CAR), and zonula occludens-1 (ZO-1) have been studied in our laboratory and are reviewed in detail below.

Plakoglobin

Recessive missense mutations or deletions in the *JUP* gene, encoding junction plakoglobin (hereafter referred to as plakoglobin), also known as γ -catenin, have been shown to be causative for Naxos disease (373–381), a recessive form of ACM with palmoplantar keratoderma and woolly hair, whereas dominant missense mutations or insertions/deletions have mostly been associated with ACM alone (382–388). Furthermore, reduced plakoglobin localization at the ICD in ACM patients regardless of the causative mutation has been reported (389, 390). Plakoglobin is an 82-kDa protein belonging to the armadillo protein family and a close homolog of β -catenin. Although β -catenin is present only in the fascia adherens junction, plakoglobin is present both in the desmosome and the fascia adherens junction of the ICD (reviewed in Ref. 391). At the fascia adherens junction, plakoglobin binds to N-cadherin (392, 393) and α -catenin (393–395), indirectly linking cadherin to the actin cytoskeleton,

whereas at the desmosome it binds to desmoglein (396), desmocollin (397), and desmoplakin (398).

Global KO of plakoglobin resulted in embryonic lethality from E10.5 due to severe cardiac dysfunction, associated with the absence of desmosomes and alterations in the fascia adherens junctions, often resulting in cardiac rupture (399, 400). Furthermore, some animals with a different genetic background, surviving until around the time of birth, showed skin blistering and subcorneal acantholysis (400). Myofibers from KO embryos were found to be less compliant, suggesting that decreased passive compliance may be responsible for the ICD alterations (401). Heterozygous plakoglobin KO mice developed late-onset ACM, characterized by right ventricular dilation and spontaneous ventricular tachycardia, which was accelerated by endurance exercise (402). In particular, heterozygous mice showed right ventricular dilation and spontaneous ventricular tachycardia but no fibrofatty replacement or ICD abnormalities. Plakoglobin hypomorphic mice with an ~60% reduction in plakoglobin expression have also been described and demonstrated to show increased β -catenin expression but otherwise normal ICD structure and cardiac function after the postnatal period, when ~50% of the hypomorphic mice died (403). Conditional ablation of plakoglobin in the heart resulted in a more severe ACM phenotype, characterized by dilation of the right atrium and ventricles, ventricular aneurisms, severe cardiac fibrosis, cardiomyocyte death, cardiomyocyte hypertrophy, loss of desmosomes, systolic dysfunction, and spontaneous ventricular arrhythmias, resulting in sudden death starting from 1 mo of age with an average life span of 4.6 mo (404). However, in contrast to human ACM patients, no adipose tissue deposition was found. Consistent with the loss of desmosomes, desmoglein-2, an interaction partner of plakoglobin, was downregulated, whereas upregulation of β -catenin was thought to compensate for the loss of plakoglobin at the fascia adherens junction without affecting Wnt/ β -catenin-mediated signaling. A similar mouse model generated by another group showed a comparable phenotype and was used in a study demonstrating that adrenergic stimulation increases cardiac myocyte cohesion, referred to as positive adhesiotropy, via PKA-mediated phosphorylation of plakoglobin at Ser665 (405). Furthermore, positive adhesiotropy was found to be associated with ultrastructural strengthening of the ICD via plakoglobin (406). Consequently, in response to β -adrenergic stimulation, plakoglobin-deficient mice showed defective myocyte cohesion and ultrastructural reorganization as well as a blunted inotropic and chronotropic response (405, 406). The same group recently demonstrated that stabilization of desmoglein-2 interactions by a specific linking peptide is sufficient to acutely rescue cardiac arrhythmia in plakoglobin KO mice as well as restore dysfunctional conduction of excitation as a result of impaired desmosome integrity (407). Inducible cardiac-specific KO of plakoglobin in adult mice resulted in a milder cardiac phenotype, starting from ~5 mo after induction, characterized by ventricular dilation, progressive loss of cardiomyocytes, inflammatory infiltration, interstitial fibrosis, cardiomyocyte hypertrophy, upregulation of hypertrophic genes, and cardiac dysfunction (408). However, no cardiac arrhythmia could be induced despite a decreased number of desmosomes, which also showed structural alterations as well as reduced expression of connexin 43 at the gap

junction. As β -catenin and β -catenin-mediated signaling were upregulated and an increased association between β -catenin and connexin 43 was found, it was hypothesized that upregulation of β -catenin can partially compensate for the absence of plakoglobin and protects from cardiac arrhythmia and sudden cardiac death (408, 409). This was confirmed in cardiac-specific double-KO mice deficient for plakoglobin and β -catenin induced at adult stage, which showed severe conduction abnormalities and spontaneous arrhythmia, resulting in sudden cardiac death between 3 and 5 mo after deletion (409) [β -catenin KO mice show no cardiac abnormalities presumably due to upregulation of plakoglobin (410)]. Consistently, double-KO mice showed loss of ICD structures, associated with a dramatic reduction in fascia adherens junction and desmosomal proteins as well as gap junction remodeling, occurring before the onset of arrhythmia.

Whereas there were no signs of adipose deposition in any of the reported plakoglobin KO mice, Tg mice overexpressing plakoglobin in cardiomyocytes showed fibrofatty infiltration and fibrosis but otherwise normal desmosome structure and cardiac function (411). The plakoglobin transgene was targeted to the nucleus, where it was found to bind to the TCF712 transcription factor and suppress Wnt/ β -catenin signaling, resulting in upregulation of adipogenic factors normally inhibited by canonical Wnt signaling. Tg mice with cardiomyocyte-specific overexpression of truncated plakoglobin, corresponding to a homozygous 2-base pair deletion, causative for Naxos disease (377), showed an amount of fibrofatty infiltration and fibrosis similar to plakoglobin WT Tg mice but demonstrated increased LV weight-to-body weight ratio, LV dilation, and systolic dysfunction (412). Furthermore, although the WT plakoglobin transgene was detected both in the nucleus and the desmosome, mutant plakoglobin was absent from the desmosome and showed reduced binding to the desmosomal proteins desmoplakin and desmoglein-2. To exactly mimic the human Naxos disease mutation, KI mice for the 2-bp deletion were generated (413). KI mice died at postnatal day 1 and showed significant downregulation of plakoglobin due to nonsense-mediated RNA decay, associated with downregulation of desmosomal proteins and upregulation of β -catenin, but otherwise no cardiac abnormalities, similar to the phenotype of plakoglobin hypomorphic mice (403, 413). In contrast, an engineered mouse model expressing mutant plakoglobin at WT levels showed no cardiac morphological or functional alterations, indicating that the clinical phenotype of patients with Naxos disease is due to loss of function and suggesting that increasing levels of truncated or WT plakoglobin may be used as a potential therapeutic approach for Naxos disease (413). No evidence of truncated plakoglobin was found, suggesting that the nuclear localization of plakoglobin in Tg mice was a result of the nonphysiological overexpression.

CAR

CXADR, encoding the coxsackievirus and adenovirus receptor (CAR), has not been directly linked to cardiac disease, but in a genome-wide association study a single-nucleotide polymorphism (SNP) at locus 21q21 (rs2824292) in the vicinity of *CXADR* has been strongly associated with ventricular fibrillation after acute myocardial infarction, a leading cause

of sudden cardiac death (414). The risk allele was associated with lower *CXADR* mRNA levels in human LV biopsies, suggesting that reduced CAR levels predispose to ischemia-induced ventricular fibrillation (415). In another study, a nonsynonymous SNP (rs437440) in the *CXADR* gene was associated with elevated systolic and diastolic blood pressure (416). CAR is a 46-kDa transmembrane cell adhesion protein with a well-known role as a viral receptor involved in the pathogenesis of viral myocarditis (417, 418). CAR is strongly expressed during development but downregulated in the adult heart, where it is present only in interstitial cells. However, it was reported to be reexpressed in the ICD and sarcolemma in human DCM (419). Furthermore, increased CAR transcript levels were reported in patients with DCM, ischemic cardiomyopathy, and mitral valve disease (420), and CAR was found to be upregulated in rat models of cardiac injury (421) and inflammation (422). Consistent with its location at the ICD, CAR has been reported to interact with ZO-1, β -catenin, and connexin 45 (413, 423, 424).

Global KO of CAR resulted in embryonic lethality at \sim E11.5–E13.5, as independently demonstrated by three different groups using slightly different targeting approaches (425–427). In all three mouse-lines, cardiac embryonic development was delayed. Asher et al. (427) reported myocardial apoptosis and wall thinning, resulting in myocardial rupture and thoracic hemorrhage. In contrast, the other two groups did not observe apoptosis or hemorrhaging (425, 426), although Dorner et al. (426) reported more frequent hemorrhaging during embryo preparation. Both Dorner et al. (426) and Chen et al. (425) reported reduced myofibrillar organization, thickness, and density as well as dilation of cardiac veins. However, although Dorner et al. found no alterations in proliferation (426), Chen et al. reported increased cardiomyocyte proliferation, resulting in hyperplasia of the LV (425). Furthermore, abnormal junctions between LV cardiomyocytes were observed. Chen et al. also found absence of the sinoatrial valve (425), whereas Dorner et al. reported enlarged endocardial cushions and abnormal atrioventricular canal formation, consistent with a developmental delay (426). The different phenotypes of the mice were suggested to be related to different genetic backgrounds and targeting strategies (425). Chen et al. (425) also generated two cardiomyocyte-specific CAR KO mouse-lines by breeding *Car* floxed mice with *Tnnt2-Cre* mice and *Myh6-Cre* mice. Cardiomyocyte-specific CAR ablation by E9.5 (using *Tnnt2-Cre* mice) (425, 428) resulted in embryonic lethality and a phenotype similar to global CAR KO mice, whereas mice with cardiomyocyte-specific CAR ablation from \sim E11 (using *Myh6-Cre* mice) (425) survived to adulthood and showed no obvious cardiac abnormalities. This suggests that CAR is essential for normal cardiac development at a specific temporal window, after which it is no longer required for survival. Both global CAR KO mice and cardiomyocyte-specific KO mice (using *Myh6-Cre* mice) were later generated by Lim et al. (429). Consistent with previous reports, global KO mice died embryonically, often with hemorrhage and pericardial effusion, whereas no evidence of structural defects, hypertrophy, or ventricular wall thinning was found (429). Cardiomyocyte-specific KO mice were viable but showed late-onset cardiomyopathy starting from \sim 5 mo of age, characterized by reduced systolic function, fibrosis, and ICD

alterations but otherwise normal cardiac dimensions. This was preceded by a first-degree or complete block of atrioventricular (AV) conduction as well as loss of connexin 45 from the cell-cell junctions of the AV node, where CAR is normally present. Furthermore, reduced expression levels and localization of β -catenin and ZO-1 at the ICD were observed. Similarly, prolonged PR intervals were observed in the embryonic heart of global CAR KO mice, consistent with a first-degree AV block. In another study, cardiomyocyte-specific KO mice generated with *Myh6-Cre* mice were found to be embryonic lethal, so to circumvent the embryonic lethality inducible cardiomyocyte-specific CAR KO mice were generated (430). Similar to observations by Lim et al., increasing prolongation of AV conduction was observed from 2 wk after induction, resulting in a complete AV block by 6 wk after induction (430). This was often associated with altered sinus node function, as indicated by accelerated junctional rhythms and the presence of sinus node tachycardia and bradycardia in some mice. Furthermore, reduced protein levels of connexin 43 and 45 were found. Similarly, a complete AV block was reported in global inducible CAR KO mice 24 wk after induction, which also showed ICD abnormalities, including a disconnection between myofilaments, the presence of big vacuoles, and wider fascia adherens junctions (431). Since elevated CAR expression has been reported in heart failure patients (419, 420), Tg mice overexpressing CAR in cardiomyocytes were studied (432). Tg mice died before 4 wk after birth with enlarged, misshaped, and disorganized cardiomyocytes; increased heart size; dilated ventricles; as well as ICD alterations, including disrupted fascia adherens junctions. This was associated with altered N-cadherin expression as well as translocation of β -catenin to the nucleus and consequent activation of c-Myc, a major downstream target of β -catenin involved in cardiac hypertrophy.

ZO-1

Missense mutations in the *TJPI* gene, encoding zonula occludens-1 (ZO-1), also known as tight junction protein 1 (TJPI), have recently been linked to ACM (433), although further studies are required to establish whether *TJPI* is a novel disease gene. ZO-1 is a member of the membrane-associated guanylate kinase (MAGUK) family and is expressed at the tight junction in different cell types and tissues (434). In the heart, ZO-1 is localized at the adherens and gap junctions of the ICD (7, 435–437). ZO-1 is a 220-kDa protein, containing three PDZ domains, a proline-rich SH3-domain binding region, and a catalytically inactive guanylate kinase domain. In the heart, ZO-1 interacts with F-actin (438–440) as well as several proteins at the ICD, including α -catenin (438), connexin 43 (435, 441, 442), connexin 45 (443, 444), CAR (423), and vinculin (445). In particular, through its link to connexin 43, ZO-1 has been shown to play a role in regulating the localization, size, number, and distribution of gap junctions (437, 446–448). ZO-1 was found to be upregulated in heart failure patients and to show stronger colocalization and increased interaction with connexin 43 (436), based on which a role of ZO-1 in the connexin 43 downregulation and decreased size of gap junctions observed in failing human heart was proposed. In contrast, other studies reported reduced ZO-1 expression and loss of ZO-1 from the ICD in failing human

hearts (449, 450), warranting further studies on larger patient groups.

Global KO of ZO-1 in mouse resulted in embryonic lethality by E11.5, associated with developmental delay and severe growth defects from E8.5. Furthermore, KO embryos exhibited extensive apoptosis in the notochord, neural tube area, and allantois as well as extraembryonic defects, including impaired yolk sac angiogenesis and defective chorioallantoic fusion. To circumvent the embryonic lethality, conditional (451) and inducible (452) cardiomyocyte-specific ZO-1 KO mice were generated. Conditional KO of ZO-1 in the heart did not affect ventricular structure, morphology, or function either under basal conditions or in response to mechanical pressure overload. However, atrial mass was increased and cardiomyocyte-specific ZO-1 KO mice exhibited various degrees of high-grade AV block, including complete block. Upregulation of ZO-2 appeared to compensate for ZO-1 deficiency in the ventricles, whereas its expression was unaltered in the AV node. While the expression and localization of ZO-1-associated proteins were not affected in the ventricles, reduced connexin 45 and CAR expression were found in atrial tissue and expression levels of connexin 45 and the cardiac sodium channel $\text{Na}_v1.5$ were reduced in AV nodal cells. As in conditional cardiomyocyte-specific ZO-1 KO mice (451), induction of ZO-1 KO in adult mice resulted in AV block. This was associated with reduced expression and localization of connexin 40 and CAR at the ICD of the AV node (452). Furthermore, in contrast to observations in conditional ZO-1 mice, the localization of connexin 43 and CAR at the ICD in ventricular cardiomyocytes was decreased and a modest reduction in systolic function in the absence of histological abnormalities was observed from 10 days after induction. Analysis of inducible conduction system-specific (using *Hcn4-Cre*) and AV bundle-His-Purkinje-specific (using *Kcne1-Cre*) ZO-1 KO mice revealed that ZO-1 expression in the conduction system proximal to the His bundle is required for conduction and that myocardial dysfunction is unrelated to the conduction system defects in cardiomyocyte-specific ZO-1 KO mice (452). The relevance of ZO-1 in the human AV node was demonstrated by the colocalization of ZO-1 with connexin 40 in ICDs of the AV node and atria but not ventricles in human biopsies.

COSTAMERE

Costameres are striated muscle-specific structures connecting the contractile apparatus to the sarcolemma, structurally and functionally resembling focal adhesions (56, 453, 454). Costameres physically link Z-lines to the extracellular matrix (ECM) and act as major mechanotransduction hubs, bidirectionally transmitting force signals between the sarcomere and the ECM. The dystrophin-glycoprotein (DGC) and vinculin-talin-integrin complexes are the two major components of costameres. Core components of the DGC include extracellular (α -dystroglycan), transmembrane (sarcoglycans, β -dystroglycan, sarcospan), and cytosolic (dystrophin, dystrobrevin, syntrophins) compartments (reviewed in Refs. 455–457). Dystrophin is a key component, physically connecting sarcomeres to the DGC via its bivalent interaction with β -dystroglycan (458) and actin filaments (459, 460). Moreover, through interaction with ankyrins, dystrophin

connects costameres to the spectrin-based filament network (461). The vinculin-talin-kindlin-integrin complex tethers membrane-spanning integrins to the actin cytoskeleton via several interconnected mechanisms (see Fig. 1) (462–465). Talin connects integrins to the actin cytoskeleton via vinculin-mediated interaction with α -actinin and recruits focal adhesion kinase (FAK) to mediate downstream integrin signaling. Moreover, integrins are connected to the actin cytoskeleton via the ILK-PINCH-parvin complex (see Fig. 1) (466–470). In addition to the core components, numerous other proteins associate with the DGC and vinculin-talin-integrin complex at the costamere (reviewed in Refs. 55, 56). One of them, filamin C, has been shown to associate both with β_1 -integrin and the DGC via interaction with sarcoglycans (reviewed in Ref. 471), proving a link to the Z-line, where it interacts with several proteins.

The clinical relevance of mutations in components of the DGC is exemplified by mutations in *DMD* (dystrophin), which are causative for X-linked Duchenne and Becker muscular dystrophy, also associated with DCM, which is a main cause of death in these patients (472, 473). A significant prevalence of DCM in sarcoglycanopathy patients, primarily manifesting as skeletal muscle myopathy, has also been reported (474, 475), and mutations in *SGCD* (δ -sarcoglycan) have also been associated with isolated DCM (476, 477). Furthermore, mutations in *DTNA* (α -dystrobrevin) have been associated with LVNC (62, 478, 479), and a *STNAI* (α 1-syntrophin) mutation has been linked to long QT syndrome (480). Also, mutations in genes encoding other components of the costamere have been associated with disease, including *VCL* (vinculin), associated with DCM and HCM (481), *ILK* (integrin-linked kinase), linked to DCM and ACM (482, 483), and *FLNC* (filamin C) (484), associated with various types of cardiomyopathy. Our laboratory has contributed to the understanding of the cardiac functions of vinculin, the ILK-PINCH-parvin complex, and filamin C as reviewed in detail below.

Vinculin

Mutations in the *VCL* gene, encoding vinculin and metavinculin, have been associated with both DCM (485, 486) and HCM (486–489). Despite low penetrance, *VCL* loss-of-function variants were enriched in probands with primarily pediatric-onset DCM, indicating that heterozygous loss of function of *VCL* alone is insufficient to cause cardiomyopathy but that these variants contribute to disease risk (481). Indeed, it was reported that cosegregation of heterozygous variants in *VCL* and sarcomeric genes, such as *TPMI* and *MYBPC3*, causes or modulates the severity of cardiomyopathy (490, 491). Vinculin is a ubiquitously expressed 117-kDa membrane-associated scaffolding protein present at the costamere and fascia adherens junction of the ICD (492, 493). Vinculin contains a globular head, a flexible proline-rich linker, and a tail and interacts with numerous interaction partners through its three regions. The head domain binds to talin (494–496), α -actinin (497, 498), α -catenin (499, 500), β -catenin (501, 502), and ZO-1 (445); the proline-rich region interacts with vasodilator-stimulated phosphoprotein (VASP) (503), vinexin/SORBS3 (504), ponsin/CAP (505), and the Arp2/3 complex (506); and the tail region binds to phosphatidylinositol 4,5-bisphosphate (PIP₂) (507, 508), paxillin (509, 510),

raver1 (511), α -synemin (512), PKC α (513), and F-actin (514–517). Through autoinhibitory interaction between the head and tail domains vinculin is kept in an inactive state, occluding ligand binding, and is believed to get activated through a conformational change induced by synergistic ligand binding, phosphorylation, and/or force (reviewed in Ref. 518). In cardiomyocytes, vinculin links integrins in costameres and cadherins in the fascia adherens junction of the ICD to the actin cytoskeleton through its interactions with talin, catenins, and α -actinin (519–522). Moreover, via interaction with ZO-1, vinculin stabilizes connexin 43-containing gap junctions at the ICD (445). A larger splice isoform of vinculin, named metavinculin (145 kDa), containing a 68-amino acid insertion within the actin-binding tail domain, is specifically expressed in cardiac and smooth muscle (493, 523–526). Both vinculin and metavinculin bind to F-actin, but whereas vinculin is able to bundle F-actin through dimerization, metavinculin inhibits vinculin-mediated F-actin bundling (527, 528) and mutations within the 68-residue insertion in the metavinculin tail domain have consequently been found to promote the formation of large disorganized actin assemblies in vitro (487). Functionally, metavinculin is capable of sustaining higher mechanical forces, but a smaller fraction of metavinculin molecules is engaged in mechanotransduction compared with vinculin (529).

Global loss of vinculin caused embryonically lethality by E8–E10 with severe cardiac hypoplasia evident at E9.5, whereas heterozygous vinculin KO animals were viable and demonstrated haploinsufficiency (530). Heterozygous vinculin KO mice had normal cardiac contractility but showed widened QRS complexes and ICD abnormalities. Furthermore, when subjected to mechanical pressure overload by TAC, they showed increased lethality and systolic dysfunction associated with Z-line misalignment and abnormal myofibril anchorage at the ICD (531). Cardiomyocyte-specific KO of vinculin led to sudden arrhythmic death in ~49% of KO mice before 3 mo of age due to conduction abnormalities and ventricular tachycardia, whereas surviving mice developed DCM and died by 6 mo of age (532). Ultrastructural analyses showed abnormal fascia adherens junctions and ICD structure, associated with reduced cadherin and β_1 -integrin levels as well as laterization of connexin 43 (532). In contrast, global metavinculin-specific KO mice were born at expected Mendelian ratios, exhibited unaltered cardiac structure, and showed a normal hypertrophic response to TAC (529), indicating that metavinculin is not essential for proper cardiac function.

Integrin-Linked Kinase

Missense mutations in the *ILK* gene, encoding integrin-linked kinase (ILK), a central component of the costameric ILK-PINCH-parvin complex, have been associated with ACM (533). Furthermore, rare ILK variants with unknown pathogenicity have been reported in DCM patients (483, 534, 535). In addition, ILK was reported to be upregulated in hypertrophic ventricles from patients with outflow tract obstruction (536). The ILK-PINCH-parvin complex connects β -integrins to the actin cytoskeleton, acting as a regulator of microtubule dynamics, gene transcription, and cell-cell adhesion (537, 538). ILK is a 55-kDa protein, consisting of four NH₂-terminal ankyrin repeats, a central phosphoinositide-binding pleckstrin

homology domain, and a COOH-terminal atypical kinase domain (539). The ILK ankyrin repeats bind to PINCH-1/2/LIMS1/2 (540, 541), thymosin β 4 (542), EPHA1 (543), and ILKAP (544, 545), whereas the ILK kinase domain interacts with β_1 -integrin (546), β_3 -integrin (547), α -parvin/CH-ILKBP/actopaxin (548, 549), β -parvin/affixin (550), paxillin (551–553), kindlin-2 (554, 555), rictor (556), AKT1(557), and SRC (558). ILK was initially thought to act as an active protein kinase capable of phosphotransfer (546, 559). However, follow-up studies demonstrated that ILK is a catalytically inactive pseudokinase, although this has been a matter of debate (560, 561). In-line with this, effects of supposedly “kinase-dead” ILK mutants in mouse models were shown to be caused by destabilization of the ILK pseudokinase domain structure and impaired protein-protein interactions, and not the loss of its putative catalytic activity (561–563).

Global KO of ILK led to death at the preimplantation stage before heart formation (564). Striated muscle-specific KO of ILK caused severe DCM, resulting in death within 5–18 wk of age. This was accompanied by structural remodeling, fibrosis, downregulation of ion channels, and strong arrhythmogenicity as well as reduced AKT and β_1 -integrin/FAK signaling (565, 566). Homozygous KI mice carrying putative constitutive active (p.S343A) or activation-resistant (p.R211A) ILK mutations in the presumed ILK kinase domain were normal and did not show changes in AKT or GSK-3 β phosphorylation (567), consistent with the notion that ILK is a pseudokinase. Tg mice overexpressing WT ILK or the ILK p.S343A mutant in cardiomyocytes developed a compensated form of cardiac hypertrophy, characterized by preserved systolic and diastolic function and the absence of fibrosis (536). In contrast, Tg mice with cardiomyocyte-specific overexpression of the ILK p.R211A mutant, incapable of binding to phosphoinositol (557) and α -parvin (548, 568), did not develop cardiac hypertrophy and showed a blunted hypertrophic response to ANG II (536). Furthermore, ILK-R211A Tg mice showed HSPA-dependent cardioprotection against myocardial infarction-induced injury, reducing infarct size and cardiac dysfunction (569). Similarly, transgenic expression of ILK p.R211A protected against doxorubicin-induced cardiotoxicity, limiting apoptosis, maintaining sarcomeric structure, and preserving cardiac function through modulation of SERCA2 and phospholamban function via a scaffolding mechanism (570). Noteworthy, the progenitor cell marker islet-1 was expressed in the heart of ILK-R211A Tg mice, supporting a cardiomyogenic role of ILK (571). Taken together, biochemical and mouse studies indicate that ILK is essential for normal cardiac function and development, which is not mediated via phosphorylation. Further studies are required to identify through which noncatalytic mechanisms ILK governs cardiac homeostasis.

Filamin C

Mutations in the *FLNC* gene have been associated with all major types of human cardiomyopathy, including HCM, DCM, RCM, and ACM. Clinically, filamin C-truncating mutations have been associated with DCM and ACM with an increased risk of sudden cardiac death (482, 572–581), whereas missense mutations have predominantly been linked to HCM and RCM (573, 582–586). Moreover, cardiac conduction defects and

hypertrophy are observed in one-third of filamin C-related skeletal myopathy cases (587, 588). Although protein aggregates are typical pathological findings in skeletal muscle biopsies in patients with filamin C-related skeletal myopathy, no aggregates have been found in hearts of DCM patients carrying *FLNC* mutations (579, 589). Because of the high prevalence of rare *FLNC* variants in the general population, variable penetrance of HCM mutations, and lack of mechanistic insights, more studies are required to identify the true pathogenicity of *FLNC* variants (590, 591). Filamins are large, homodimeric actin-binding and cross-linking proteins (592–594). Filamin C is a 290-kDa protein specifically expressed in striated muscle (595, 596) and composed of an NH₂-terminal paired actin-binding calponin-homology (CH) domain, followed by 24 Ig-like domains, structured into two rod regions (594, 596). The relative flexibility of the two rod regions regulates the spatial characteristics of F-actin cross linking via multiple molecular interactions (597). The last COOH-terminal Ig domain, Ig24, mediates dimerization of filamin C, which is required for its actin cross-linking activity (598, 599). In cardiomyocytes, filamin C is localized at the Z-line, costamere, and ICD (595, 600). In the Z-line, filamin C interacts with myotilin (601, 602), members of the calsarcin/FATZ/myozenin family (75, 602–604), myopodin (605), nebulin (151), and aciculin/phosphoglucomutase 5 (PGM5) (606); at the costamere, it binds to sarco-glycans (596), β_1 -integrin (607), and ponsin/CAP/SORBS1 (608); and at the ICD, it interacts with NRAP (609), Xin/XIRP1/CMYA1 (610), and XIRP2/CMYA3 (610). These multifaceted protein associations underlie the role of filamin C in the organization and stabilization of the cardiomyocyte cytoskeleton (471, 611, 612).

Deletion of the last 8 exons (exons 41–48) of *Flnc* in mouse resulted in perinatal lethality due to respiratory failure and severe defects in skeletal myogenesis but no overt cardiac phenotype (613). The introduced deletion resulted in a hypomorphic allele, as truncated filamin C was still expressed at low levels in mutant mice. A true *Flnc* null allele was generated by deletion of exons 9–13, causing a frameshift in the *Flnc* coding sequence and subsequent loss of the protein (614). Both global and cardiomyocyte-specific loss of filamin C led to embryonic lethality at E10.5 (614), accompanied by severe chest edema (unpublished data). Cardiomyocyte-specific ablation of filamin C in adult animals led to rapidly progressing DCM leading to death within 2 wk, accompanied by upregulation of multiple proteins, including direct filamin C interaction partners at the costamere and ICD (614).

T-TUBULES AND SARCOPLASMIC RETICULUM

Deep invaginations in the sarcolemma, termed t-tubules, and their interaction with the sarcoplasmic reticulum (SR) in microdomains, named dyads, are essential for calcium handling and excitation-contraction coupling, governing heart contraction (reviewed in Ref. 9). At the dyads, depolymerization of the plasma membrane causes an influx of calcium through voltage-gated L-type calcium channels (LTCCs), triggering calcium release from the SR through the ryanodine receptor (RyR2) in a process called calcium-induced calcium release (CICR). The rapid increase in

intracellular calcium initiates sarcomere contraction, which is subsequently terminated by the removal of cytosolic calcium, primarily through its reuptake into the SR via the cardiac SR calcium ATPase (SERCA2), regulated by phospholamban (615) but also to a lesser extent by extrusion through the sodium/calcium exchanger (NCX) in the plasma membrane. Calsequestrin 2 (CASQ2), a calcium-binding protein located in the lumen of the SR, acts as a calcium buffer and negative regulator of RyR2 in complex with triadin and junctin to prevent spontaneous SR calcium release (616). The key interaction between LTCCs localized in the t-tubules and RyR2 on the cytosolic side of the dyad is stabilized and maintained through numerous protein-protein and protein-lipid interactions (reviewed in Refs. 8–10, 617).

Mutations in genes encoding cardiac dyad proteins directly involved in calcium handling, such as *RYR2*, *TRDN* (triadin), and *CASQ2* (calsequestrin 2), most frequently lead to an inherited arrhythmia known as catecholaminergic polymorphic ventricular tachycardia (616), which in some cases is accompanied by LVNC (618). Furthermore, *RYR2* mutations have been identified in ACM (619, 620) and DCM (621) patients, and *RYR2* loss of function mutations were recently associated with sudden cardiac death with normal exercise stress test, a novel syndrome that was named RyR2 calcium release deficiency syndrome (CRDS) (622). In addition, mutations in *PLN* (phospholamban) have been associated with DCM, HCM, and ACM (623). Mutations in genes encoding LTCC subunits have been linked to different arrhythmogenic diseases, including Brugada syndrome (*CACNA1C*, *CACNB2*, *CACNA2D1*), Timothy syndrome (*CACNA1C*), long QT syndrome (*CACNA1C*, *CACNA2D1*), and short QT syndrome (624). Moreover, mutations in *LRRK10*, encoding the LTCC auxiliary protein leucine-rich repeat containing protein 10, have been associated with DCM (625, 626). Our laboratory has contributed to the understanding of cardiac dyads by identifying the human cardiomyopathy gene *NEXN* (nexilin) as a cardiac dyad protein and creating a junctophilin-2 based murine model to study the composition of cardiac dyads, as discussed in detail below.

Junctophilin-2

Mutations in the *JPH2* gene, encoding junctophilin-2, have been found in patients with HCM (627–630) and autosomal recessive pediatric DCM (631). Junctophilin-2 is a 74-kDa protein belonging to a four-member family of human junctophilins (632). All junctophilins contain eight NH₂-terminal membrane occupation and recognition nexus (MORN) motifs, a central α -helical domain, and a COOH-terminal transmembrane region (633). Junctophilin-2 is predominantly expressed in the heart and tethers transverse tubules to the junctional SR (634), forming cardiac dyads (9, 635). The MORN motifs mediate the interaction with the plasma membrane (636), the central α -helical domain is thought to determine the 12-nm distance between the plasma membrane and the SR (637, 638), and the transmembrane region anchors junctophilin-2 to the SR membrane (638). Junctophilin-2 plays an important role in cardiomyocyte calcium homeostasis, which is partially mediated via interaction with RyR2 (639, 640), the *CACNA1C* subunit of the LTCC (641–643), the voltage-gated potassium channel

subunit K_v7.1 (KCNQ1), and the small-conductance calcium-activated potassium channel subtype 2 (KCNN2) (644) as well as the nonchannel proteins nexilin (645), caveolin-3 (646), and striated muscle enriched protein kinase (SPEG) (647). Moreover, under cardiac stress, the proteolytic fragment of junctophilin-2 translocates to the nucleus, where it acts as a transcriptional regulator, attenuating pathological remodeling in response to cardiac stress (634).

Global KO of junctophilin-2 resulted in embryonic lethality from E10.5 due to developmental cardiac failure (638). Cardiomyocytes from junctophilin-2 KO embryos isolated at E9.5 showed defective calcium handling due to physical LTCC and RyR2 uncoupling, causing impaired CICR and consequent calcium overload in the SR, leading to vacuolization. Constitutive cardiomyocyte-specific shRNA-mediated downregulation of *Jph2* led to mouse strain-specific development of DCM, accompanied by abnormal calcium transients and impaired maturation of t-tubules (648, 649). Depending on the extent of inducible junctophilin-2 downregulation, adult mice developed acute heart failure under basal conditions or exhibited enhanced cardiac vulnerability to pressure overload (640, 650). In all reported studies, adult junctophilin-2 downregulation in heart led to abnormal calcium handling (640, 650, 651). Murine KI models of *JPH2* mutations recapitulated certain features of human disease. A murine pseudo-KI (PKI) model of the *JPH2* p.E169K mutation found in a patient with early-onset paroxysmal atrial fibrillation (AF) in the context of HCM has been reported (639). The *JPH2*-E169K PKI model was generated by intercrossing cardiomyocyte-specific mutant Tg mice with a mouse-line with inducible cardiac-specific shRNA-mediated knockdown of *JPH2* (640). *JPH2*-E169K PKI mice were more susceptible to AF induction and had increased spontaneous SR calcium leak attributed to weaker binding of mutant protein to RyR2 (639). However, the HCM phenotype was not recapitulated in the *JPH2*-E169K PKI model. Using the same principle, a murine PKI model for the *JPH2* p.A399S mutation, corresponding to the human *JPH2* p.A405S mutation found in an HCM patient, was generated (628). In accordance with the clinical history of the patient, no overt calcium handling abnormalities were observed in this PKI model. However, asymmetric septal hypertrophy was observed, closely recapitulating the clinical findings. Unaltered calcium handling yet pathological septal hypertrophy in the *JPH2*-A399S PKI mouse model suggest that junctophilin-2 is involved in cardiac homeostasis independent of calcium handling. To advance the understanding of cardiac dyad molecular organization in the native context, a new mouse model was created by fusing BioID2 to the endogenous *JPH2* coding sequence (652). BioID2 is a promiscuous biotin ligase mutant used for proximity-dependent biotinylation for characterization of protein complexes (653). This approach has been proven to be more sensitive and specific than other methods applied to study the interactome of cardiac dyads and led to the identification of potential novel junctophilin-2-associated proteins that may play essential roles in cardiac dyad formation, maintenance, and function (652).

Nexilin

Mutations in the *NEXN* gene, encoding nexilin, have been associated with HCM (654) and DCM (655, 656). Nexilin is

expressed in two isoforms of ~75 and 100 kDa and predominantly expressed in cardiac and skeletal muscle as well as in smooth muscle cells (655, 657, 658). Initially identified as a cardiac Z-disk protein (655), nexilin was later found to be a component of junctional membrane complexes in cardiac dyads, where it interacts with junctophilin-2 and RyR2 (645). Furthermore, it binds to F-actin (657) and has been shown to promote actin polymerization and cell migration in smooth muscle cells (658). Nexilin was also recently reported to be reduced in human atherosclerotic plaques as well as in the serum of patients with coronary artery disease (659). Reduced nexilin levels were found to be associated with increased atherosclerosis and inflammation in atherosclerotic lesions, suggesting nexilin as a potential therapeutic target in atherosclerosis-related diseases.

Global nexilin KO mice developed rapidly progressive postnatal DCM with wall thinning, leading to death within 12 days after birth (645, 660). Furthermore, nexilin KO mice showed endocardial deposits, which were initially attributed to endomyocardial fibroelastosis (660) but later suggested to be intracardiac mural thrombi (645). Cardiomyocyte-specific nexilin KO recapitulated the phenotype, demonstrating that the phenotype of global nexilin KO mice is a direct consequence of its loss in cardiomyocytes (645). Further analyses showed failure of cardiac-specific nexilin KO mice to initiate and form t-tubules, resulting in impaired calcium handling, altered expression of calcium-related proteins, and consequent DCM (645). Ablation of nexilin in adult murine hearts, in which the t-tubular network is already formed, led to DCM accompanied by disorganization of the t-tubular network, defective calcium homeostasis, and reduced sarcomere shortening, indicating that nexilin is required for the maintenance of the t-tubular network in adult heart (661). Homozygous KI mice carrying the NEXN p.G645del mutation equivalent to the human NEXN p.G650del mutation associated with DCM developed progressive DCM, characterized by reduced t-tubular formation and disorganization of the transverse-axial tubular system (662). Levels of mutant protein in homozygous animal hearts were only 30% of WT levels, suggesting that the NEXN p.G645del mutation affects nexilin stability. However, heterozygous global nexilin KO mice or mice carrying a deletion of *Nexn* exons 3 and 4 showed normal heart function, despite expression of 50% and 20% of nexilin, respectively, compared with WT control animals (662). These findings indicate that not pure haploinsufficiency but altered functionality of mutant nexilin is driving cardiomyopathy in the NEXN-G645del KI model (662).

NUCLEUS

Nuclei in cardiomyocytes, highly specialized, mechanically active, mainly postmitotic cells, face a unique environment that requires special molecular mechanisms to govern nuclear integrity and tissue functionality. The nucleus is separated from the cytoplasm by the nuclear envelope (NE), which is composed of two lipid bilayers, the outer nuclear membrane (ONM) and the inner nuclear membrane (INM), enclosing the perinuclear space (PNS), which also contains nuclear pore complexes (NPCs) allowing bidirectional transport of macromolecules across the NE (reviewed in Refs.

663–665). The ONM is connected to the peripheral SR and the cytoskeleton, whereas the INM is supported by the nuclear lamina, a meshwork of intermediate filaments composed of A-type (A and C) and B-type (B1 and B2) lamins as well as lamina-associated proteins such as the lamin B receptor (LBR), lamina-associated polypeptide 1 and 2 (LAP1 and LAP2), emerin, and LEM domain containing 3 (LEMD3)/MAN1. The linker of nucleoskeleton and cytoskeleton (LINC) complex spans the NE and is formed by the association of nesprins at the ONM with Sad1 and UNC84 domain-containing (SUN) proteins at the INM within the PNS. SUN1/2 proteins at the INM interact with laminaA/C, emerin, luma, components of the nuclear core complex, and heterochromatin in the nucleoplasm, whereas nesprins at the ONM associate with actin filaments, microtubules, and intermediate filaments, tethering the LINC complex to the cytoskeleton. Thus, the LINC complex provides structural support to the nucleus and mechanically couples the cytoskeleton to the nuclear lamina and associated chromatin. Furthermore, increasing evidence indicates a role of the LINC as a mechanosensor, translating external mechanical forces into biochemical signals, triggering changes in NE structure and composition, chromatin organization, gene expression, and nuclear calcium handling (reviewed in Ref. 666). In addition, cardiac development, maintenance, and response to stress are dependent on cardiac transcription factors directly interacting with genomic DNA (667–670).

Because of the specialized environment of cardiac nuclei, mutations in nuclear and nucleus-associated proteins have been associated with a wide range of clinical syndromes, including cardiomyopathy. Mutations in several genes encoding proteins of the NE can cause Emery–Dreifuss Muscular Dystrophy (EDMD), associated with DCM, HCM, cardiac conduction defects, and/or arrhythmias, including *EMD* (emerin), *LMNA* (lamin A), *TMEM43* (luma), and *SYNE1/2* (nesprin-1/2) (reviewed in Refs. 664, 671). *LMNA* mutations have also been associated with DCM and congenital dystrophy with cardiac involvement (672, 673), *TMEM43* mutations with ACM (664), and *SYNE1/2* mutations with isolated DCM and ataxia with DCM (672). Mutations in SUN proteins do not appear to be a primary cause of cardiomyopathy, but SUN variants can act as disease modifiers in combination with mutations in other EDMD-associated genes (674). Mutations in *LEMD2*, encoding LEM domain nuclear envelope protein 2 located in the INM, were found to be associated with autosomal recessive juvenile-onset cataracts and arrhythmic cardiomyopathy with mild impairment of LV systolic function (675, 676). Furthermore, mutations in *TOR1AIP1* (LAP1) have been linked to dystonia and cerebellar atrophy with DCM as well as LGMD with cardiac involvement (677, 678), whereas a mutation in *TMPO2* (LAP2 α) has been associated with DCM (679). Mutations in genes encoding nucleoporins (NUPs) have also been linked to cardiovascular disease, including *NUP155* and *NUP37*, which have been associated with atrial fibrillation and sudden cardiac death (680, 681). Furthermore, mutations in proteins enclosed in the nucleoplasm, such as transcription factors, can cause cardiomyopathies. For example, biallelic truncating mutations in *ALPK3* (α -protein kinase 3), exclusively expressed in striated muscle and localized in the nucleus, where it acts as a transcriptional regulator through phosphorylation of cardiac transcriptional factors, have

been linked to severe pediatric cardiomyopathy, mostly manifesting as neonatal DCM transitioning to HCM, whereas heterozygous *ALPK3* mutations have been associated with HCM (reviewed in Ref. 623). Key components of the LINC complex, nesprins and luma, as well as the transcriptional regulators of cardiac development and function, *TBX20* and *PRDM16*, have been extensively studied in our laboratory and are reviewed in detail below.

Nesprin-1 and Nesprin-2

Gene mutations in *SYNE1* and *SYNE2*, encoding nuclear envelope spectrin repeat proteins Nesprin-1 and Nesprin-2, respectively, lead to nesprinopathies (682). Nesprinopathies manifest as isolated DCM (680, 683–685), EDMD with DCM (680, 682, 686, 687) or HCM (688), or ataxia with DCM (689). Nesprins are components of the LINC complex connecting the nuclear lamina to the cytoskeleton and belong to a ubiquitously expressed four-member family of mammalian spectrin repeat proteins of which Nesprin-1, -2, and -3 are expressed in the heart (689). Nesprin-1 and -2 exist in numerous isoforms due to variable RNA processing, resulting in alternative transcription initiation, termination, and splicing (690). The largest nesprin-1 and -2 isoforms, named “giant,” are 1.01 MDa and 796 kDa in size, respectively, and located at the ONM (691, 692). Giant nesprins contain two NH₂-terminal calponin-homology (CH) domains that bind to F-actin (693), a central spectrin repeat-containing rod domain of variable length, and a COOH-terminal transmembrane Klarsicht/Anc/Syne homology (KASH) domain, which anchors nesprins to the NE via interaction with the INM proteins *SUN1* and *SUN2* across the PNS (694, 695), forming the LINC complex. The smaller nesprin-1/2 isoforms lack either the NH₂-terminal CH domains, the COOH-terminal KASH domain, or both, and include a variable number of spectrin repeats (690, 696, 697). Although giant nesprin-1/2 isoforms are ubiquitously expressed, many smaller isoforms have tissue-specific expression and are located at distinct cellular locations, with some specifically expressed in cardiac and/or skeletal muscle (e.g., nesprin-1 α 2, -2 α 1, and -2 ϵ 2) (696). Importantly, smaller nesprin-1/2 isoforms are found at the INM, where they directly interact with emerin, lamin A/C, and *SUN1/2* through their COOH-terminal spectrin repeats (698–702). Furthermore, nesprin-1 α was found to dimerize and bind to muscle A-kinase anchoring protein (mAKAP/AKAP6) through its NH₂-terminal spectrin domains, targeting mAKAP to the NE (701, 703). The COOH-terminal region of nesprin-1/2 was also shown to bind to α -N-catenin (704) as well as kinesin light chains (KLC)-1/2 in skeletal muscle cells, implicating nesprins in nuclear positioning (705–707).

Genetic mouse models have revealed that the cardiac functions of nesprin-1 and nesprin-2 largely overlap and that the two proteins to a great extent can compensate for the loss of each other. Mice in which the nesprin-1 KASH domain has been replaced by an unrelated sequence of 61 amino acids (nesprin-1^{rKASH} mice), showed 50% perinatal mortality (683, 708), and the surviving mice developed an EDMD-like phenotype. At ~1 yr of age, the mice developed cardiac systolic dysfunction and cardiac conduction defects, preceded by the presence of elongated nuclei with irregular shape and reduced heterochromatin. However, although the interaction

with *SUN* proteins was disturbed, the location of LINC proteins at the NE was unaffected. In contrast to the nesprin-1^{rKASH} model, deletion of the KASH domains of either nesprin-1 (nesprin-1^{AKASH}) or nesprin-2 (nesprin-2^{AKASH}) did not affect viability (709), possibly because of the expression of nesprin-1/2 KASH-less isoforms (710). On the other hand, nesprin-1^{AKASH} and nesprin-2^{AKASH} double mutants died of respiratory failure within 20 min of birth (709). Similar to the nesprin-1^{rKASH} model, global KO of nesprin-1 resulted in 60% perinatal lethality and severe skeletal muscle defects, including defects in nuclear positioning and anchorage (710). However, no cardiac pathological phenotype was observed up to 12 mo of age. Global double-KO mice for nesprin-1 and the intermediate filament protein desmin, which like nesprins has been demonstrated to play a role in nuclear anchorage and positioning, showed increased mortality and a more severe dystrophic phenotype compared with single-KO mice (711). This was associated with more severe nuclear positioning and anchorage defects as well as decreased nuclear deformation under biomechanical stretch, i.e., cytoskeletal-nuclear strain transmission, compared with single-KO mice, suggesting that nesprin-1 and desmin play partially redundant roles in skeletal muscle nuclei anchorage in skeletal muscle. Through deletion of the nesprin-1 CH domain in mouse it was later shown that the nesprin-1 giant isoform is dispensable for postnatal viability and nuclear positioning, whereas nesprin-1 α 2 KO mice showed a phenotype similar to nesprin-1 KO mice, demonstrating that this isoform is essential for postnatal viability and skeletal muscle function (712). In contrast to the severe effect of nesprin-1 ablation, global nesprin-2 KO mice showed no defects in either cardiac or skeletal muscle (713). As early lethality of nesprin-1^{AKASH} and nesprin-2^{AKASH} double mutants (709) had previously precluded the analysis of cardiac function, nesprin-1 and nesprin-2 double-KO mice with cardiac-specific nesprin-1 deletion were generated (713). At 10 wk of age, double-KO mice showed reduced wall thickness and systolic dysfunction, associated with fibrosis, apoptosis, reexpression of fetal genes, and mislocalization of emerin and lamin A/C from the NE. Furthermore, changes in nuclear positioning, morphology, and heterochromatin localization were observed, which was also found to a lesser extent in single-KO mice (710, 713). Noteworthy, double-KO mice showed an impaired biomechanical gene response upon application of strain, which was also partially blunted in single-KO mice (713). This demonstrated that nesprin-1 and -2 show partial functional redundancy in the heart. Interestingly, in contrast to nesprin-2 KO mice, showing no obviously pathological phenotype, RNA interference-mediated knockdown of nesprin-2 targeting both the NH₂- and COOH-terminal-encoding parts in mice resulted in early embryonic lethality, with no knockdown embryos found by E13 (714). The authors speculated that certain isoforms may not have been targeted in the conventional nesprin-2 KO mice, whereas loss of all isoforms was achieved by the shRNA-based approach, leading to the severe embryonic phenotype not observed in other models. However, this remains to be proven.

Luma

Mutations in *TMEM43*, encoding luma, an evolutionarily conserved, ubiquitously expressed NE protein, have been

linked to ACM (715–721) and EDMD with cardiac arrhythmias (722). Notably, a recent international evidence-based reappraisal of genes associated with ACM classified the luma p.S358L mutation as a definite cause of cardiomyopathy (723). Luma is a 45-kDa protein, containing four transmembrane helices and a “domain of unknown function,” DUF1625, located between the third and fourth helix, which comprises about two-thirds of the protein and might act as a tetraspanin-like membrane organizer (724, 725). Luma is located in the INM, where it interacts with emerin, lamin A/C, lamin B1, and SUN2 (722, 724). Furthermore, several potential direct or indirect binding partners were identified by coimmunoprecipitation-coupled mass spectrometry, including β -actin (726).

Unexpectedly, global luma KO mice in the C57BL/6J background were indistinguishable from WT control mice both under basal conditions and in response to mechanical pressure overload (727). In contrast, heterozygous cardiomyocyte-specific luma KO mice in a mixed 129/C57BL/6N background developed late-onset cardiomyopathy, characterized by LV dilation, systolic dysfunction, increased cardiomyocyte size, fibrofatty infiltration, and apoptosis (728). This was associated with activation of the DNA damage response (DDR) pathway and consequent activation of TP53 and upregulation of senescence-associated secretory proteins, including transforming growth factor- β 1 (TGF- β 1). KI mice for the luma p.S358L mutation were generated by two different laboratories. In one study, homozygous luma-S358L KI mice in the C57BL/6J genetic background showed no changes in cardiac function under basal conditions up to 12 mo of age (727), whereas in another study 8-wk-old heterozygous luma-S358L KI mice in a mixed 129/C57BL/6 genetic background were found to show an ACM-like phenotype, characterized by LV dilation, posterior wall thinning, and fibrofatty infiltration, although electrocardiographic abnormalities were not detected (729). In particular, activation of the nuclear factor- κ B (NF- κ B)-TGF- β signaling cascade was found to promote cardiac fibrosis. A Tg mouse model overexpressing the luma p.S358L mutant in cardiomyocytes more closely recapitulated human ACM, developing progressive ventricular dilation, systolic dysfunction, cardiac conduction defects, apoptosis, cardiomyocyte death, and fibrofatty replacement, leading to premature death within 6 mo after birth (726). The mutant protein was found to display partial delocalization from the NE to the cytoplasm as well as reduced interaction with emerin and β -actin. In addition, luma-S358L Tg mice showed reduced AKT activity and consequent glycogen synthase kinase-3 β (GSK3 β) activation and inhibition of β -catenin-dependent transcription. Importantly, although inhibition of fibrosis had no effect on cardiac function in luma-S358L Tg mice, inhibition of GSK3 β improved cardiac function and survival, suggesting GSK3 β as a novel therapeutic target for ACM (726).

TBX20

Mutations in the *TBX20* gene, encoding a member of the T-box family of transcription factors, have been shown to be responsible for congenital heart diseases including septation and cardiac valve defects (730–732). Furthermore, *TBX20* mutations have been associated with DCM (731, 733, 734) and LVNC (735). In addition, increased *TBX20* RNA levels were found in myocardial biopsies from patients with idiopathic DCM and shown to negatively correlate with LV

function (736). *TBX20* is a 49-kDa highly evolutionarily conserved protein consisting of a single centrally located T-box domain and expressed in almost all cardiac cell-lineages (737). *TBX20* plays critical roles during early heart development, and numerous studies have identified *TBX20* as a key modulator of cardiac gene expression programs mechanistically linked to other cardiac transcription factors, including *Nkx2.5*, *GATA4*, *GATA5* (738), *ISL1*, *MEF2C* (739), *COUP-TFII* (740), *TBX5* (741), *CASZ1* (742), *P21*, *MEIS1*, and *BTG2* (743). In cardiomyocytes *TBX20* regulates the expression of key potassium and calcium channels, other ion transporters, gap junction components, and genes involved in cell cycle and growth (743–745).

Global *TBX20* KO in mice resulted in embryonic lethality around E10.5, accompanied by severe hypoplastic ventricles and defects in cardiac looping (746–748). Similarly, complete *Tbx20* knockdown by RNA interference in mouse embryos resulted in defective cardiac morphogenesis, whereas partial *TBX20* inhibition led to congenital heart defects, including impaired outflow tract septation, hypoplasia of the right ventricle, and defective valve formation (739). Ablation of *TBX20* specifically in the atrioventricular canal (AVC) and outflow tract (OFT) of the early heart tube through crossing of *Tbx20* floxed mice with *Tbx2-Cre* mice resulted in embryonic lethality by E10.5 (749). The mutant embryos showed failure to form the AVC constriction and loss of AVC cushion mesenchymal cells, demonstrating an essential role of *TBX20* for early AVC patterning and epithelial-mesenchymal transition (EMT). This effect was partially mediated through its downstream target *BMP2*, as reexpression of *BMP2*, which was dramatically decreased in mutant embryos, rescued the EMT defects. Specific deletion of *TBX20* in the endocardium and cushion mesenchyme through *Tie2-Cre*-mediated targeting did not affect the number of AVC cushion mesenchymal cells, indicating that *TBX20* modulates the EMT during early AVC development (749). Similarly, *Nfatc1-Cre*-directed deletion, likewise ablating *TBX20* in the cushion endocardium and mesenchyme, did not affect EMT initiation but led to embryonic lethality between E14.5 and E16.5 due to defective valve elongation and maturation as well as atrioventricular cushion formation, associated with aberrant WNT/ β -catenin signaling and reduced ECM gene expression (750). *Tie2-Cre*-mediated *TBX20* deletion was subsequently reported to cause cardiac cushion abnormalities and septal defects, including outflow tract (OFT) septation and atrioventricular septal defects (AVSDs) as well as lack of the dorsal mesenchymal protrusion (DMP), resulting in embryonic lethality by E14.5 (751). This was associated with reduced proliferation in OFT cushions and defective endocardium-derived cell migration as a result of reduced expression of ECM and cell migration genes critical for cardiac septation. Like *Tie2-Cre*-targeted *TBX20* KO mice, *Nfatc1-Cre*-targeted *TBX20* KO mice exhibited OFT septation defects but did not show AVSDs, which could be explained by the targeting of *Tie2-Cre* but not *Nfatc1-Cre* to the endothelium adjacent to the DMP (pulmonary venous endothelium, sinus venosus, and common atrium) (751). Cardiomyocyte-specific KO of *TBX20* from around E9.5 (using *Tnnt2-Cre* mice) did not affect initiation of heart chamber formation but led to failure of cardiac chamber expansion and septal defects, associated with reduced cardiomyocyte proliferation, resulting in

embryonic lethality around E14.5 (740). Cardiomyocyte-specific ablation of TBX20 in adult mice led to cardiac death within 5–16 days after induction, associated with chamber dilation, wall thinning, contractile dysfunction, and arrhythmias (745). The observed phenotype could to a large extent be explained by dysregulation of a transcriptional network consisting of MEF2A, TEAD, CREB1, and ESRRA, regulating calcium homeostasis and ion transport in adult heart (745). Combined genome-wide chromatin immunoprecipitation and transcriptomic analyses on this model subsequently demonstrated a role of TBX20 as a transcriptional activator and repressor depending on its association with different cofactors (744). More specifically, TBX20 was found to activate genes involved in cardiac contraction and energy metabolism, whereas it represses genes involved in development and specification of noncardiac tissues and systems repressed in the heart.

Transgenic overexpression of TBX20 under the control of the *Myh6-Cre* promoter, known to mediate overexpression by ~E11 (425), resulted in partial pre- and postnatal lethality (752). Surviving mice showed reduced body size and developed early-onset DCM, associated with systolic dysfunction, septum abnormalities, and ventricular hypertrabeculation similar to the phenotype of Tg mice for bone morphogenetic protein 10 (BMP10), an upstream regulator of TBX20. Another group overexpressed TBX20 in embryonic (E9.5) and fetal (E12.5–17.5) cardiomyocytes using *Nkx2.5-Cre* and *Myh7-Cre*, respectively (753). Overexpression of TBX20 in embryonic heart resulted in growth retardation due to reduced cardiomyocyte proliferation, resulting in embryonic lethality by E10.5. In contrast, *Myh7-Cre*-mediated TBX20 overexpression in fetal cardiomyocytes resulted in increased thickness of the developing myocardium from E14.5 as well as induction of cardiomyocyte proliferation. *Myh7-Cre*-mediated TBX20 overexpression promoted cardiomyocyte proliferation also in adult heart, resulting in an increased number of small, proliferating, mononucleated cardiomyocytes with fetal-like characteristics associated with induction of BMP2/SMAD1/5/8 and PI3K/AKT/GSK3 β / β -catenin signaling (754). Induction of cardiomyocyte-specific TBX20 overexpression in adult mice likewise promoted cardiomyocyte proliferation through activation of multiple proliferative pathways and repression of negative cell cycle regulators without affecting cardiac morphology and function (743). Notably, overexpression of TBX20 in a model of myocardial infarction enhanced cardiac repair, reducing infarct size and cardiac dysfunction, resulting in significantly increased survival (743).

PRDM16

Mutations in the *PRDM16* gene, encoding PRDI-BF1 and RIZ homology (PR) domain-containing 16 (PRDM16), have been identified in patients with DCM and LVNC (755–761). Moreover, *PRDM16* variants have been linked to QRS duration (762). PRDM16, also known as MEL1, belongs to a 19-member family (in human) of PR domain-containing (PRDM) transcription regulators and histone methyltransferases, containing a conserved NH₂-terminal PR domain and a variable number of zinc fingers (52, 763–765). Human PRDM16 is expressed in four isoforms ranging from 170 to

150 kDa, a full-length isoform, a smaller isoform lacking the PR domain, and two isoforms resulting from alternative splicing of full-length PRDM16 (766, 767). All isoforms share zinc finger repeats, a proline-rich domain, a repressor domain, and a COOH-terminal acidic domain (767). Through its zinc finger domains, PRDM16 directly binds to DNA as well as interacts with various transcription factors and chromatin regulators to promote or repress gene expression depending on the biological context (766, 768, 769). In particular, PRDM16 has been found to function as a transcriptional cofactor for the transcription factors EHMT1 (770), MED1 (771, 772), PGC-1 α (773), C/EBP β (774), PPAR γ (775), SMAD (776), and SKI (777). The intrinsic histone methyltransferase activity of PRDM16 is dependent on its NH₂-terminal PR domain (778), and together with PRDM3, PRDM16 catalyzes monomethylation of lysine 9 of histone 3 (H3K9me1), ensuring mammalian heterochromatin integrity (779).

Global PRDM16 KO mice died shortly before or after birth and showed multisystemic defects, including gross cardiac ventricular hypoplasia (780–782). Cardiomyocyte-specific PRDM16 KO mice generated by crossing of *Prdm16* floxed mice with *Myh6-Cre* mice displayed increased heart weight-to-body weight ratio, longitudinal elongation of the heart, and cardiomyocyte hypertrophy, associated with ventricular fibrosis, cardiac conduction defects, and increased expression of hypertrophic markers at 5 mo of age (783). Another conditional PRDM16 KO mouse model generated using *Mesp1-Cre*, which drives Cre expression in cardiogenic progenitors (784–786), developed late-onset HCM starting from 9 mo of age, progressing to heart failure (768). This was associated with fibrosis, increased cardiomyocyte size, increased expression of hypertrophic markers, mitochondrial dysfunction, and metabolic defects. Young mice subjected to metabolic stress induced by high-fat diet developed a similar phenotype. In contrast to the two aforementioned conditional PRDM16 KO models, *Xmlc2-Cre*- and *Tnnt2-Cre*-mediated targeting, resulting in efficient PRDM16 deletion from as early as E7.5 (425, 787), resulted in biventricular noncompaction, LV-specific dilation, and cardiac contractile dysfunction, leading to death within 7 days after birth, recapitulating the LVNC phenotype of patients carrying *PRDM16* mutations (769). This was associated with reduced cardiomyocyte proliferation specifically in the LV, likely contributing to the development of LV dilation and systolic dysfunction. The failure of *Myh6-Cre*- and *Mesp1-Cre*-targeted PRDM16 KO mice to recapitulate the LVNC phenotype may be due to later developmental deletion and variable recombination efficiencies. Cardiomyocyte-specific KO of PRDM16 in adult mice did not result in any pathological cardiac phenotype, consistent with strongly decreased PRDM16 protein levels after birth, indicating that PRDM16 does not play an important role in adult cardiomyocytes. This is in accordance with the developmental origin of LVNC, which is thought to be caused by an arrest of the normal compaction of the endomyocardial layer of the heart during early embryogenesis (769). Mechanistically, PRDM16 was found to function as a transcriptional cofactor, maintaining compact myocardial cardiomyocyte identity by activating a subset of myocardial genes required for compaction while repressing trabecular genes in LV compact myocardium, at least partly through cooperation with the LV-enriched transcription

factors TBX5 and HAND1. Consequently, PRDM16 ablation in cardiomyocytes of the developing heart led to a shift in the transcriptomic identity of cardiomyocytes associated with compact myocardium to a gene signature resembling that of trabecular cardiomyocytes and/or neurons, suggesting that misspecification of compact or trabecular cardiomyocytes may be a common pathomechanism of LVNC.

CYTOSOL

The cytosol is a semifluid solution filling the interior of the cell and surrounding the various organelles and subcellular compartments (788). The cytosol is enclosed by the cell membrane and membranes of the organelles, constituting a separate cellular compartment (789). The cytosol is composed of ~70% water as well as ions (e.g., sodium, potassium, calcium, chloride, bicarbonate, magnesium, and amino acids), smaller organic molecules (e.g., glucose and other simple sugars, polysaccharides, amino acids, nucleic acids, and fatty acids), and macromolecules (e.g., proteins) (790). The cytosol also contains protein complexes, for example, of enzymes involved in the same metabolic pathway (791) as well as protein compartments, such as the proteasome, which forms an enclosed compartment containing proteases degrading cytosolic proteins (792, 793). The cytosol allows for free movement of ions and molecules across the cell, and a major function of the cytosol is to transport metabolites from their site of production to where they are needed (794). Furthermore, molecules taken up by the cell or being secreted are transported through the cytosol in membrane-bound vesicles moved along the cytoskeleton by microtubule-based motor proteins (795, 796). Other important processes occurring in the cytosol include metabolic processes such as protein synthesis and glycolysis, cell division through mitosis, as well as signal transduction from the sarcolemma to the different sites within the cell (797–799). Furthermore, differences in concentrations between ions in the cytosol and the organelles or extracellular fluid are essential for many cellular processes, including cell signaling, osmosis, and cellular excitability, such as CICR triggering muscle contraction (800, 801).

Several cytosolic proteins have been associated with cardiomyopathy (21). Mutations in BAG3, involved in protein quality control, have been associated with DCM, HCM, and RCM (802, 803), whereas mutations in the eukaryotic elongation factor 1 alpha 2 (eEF1A2) have been linked to neurological disease associated with DCM (804–806). Both proteins have recently been studied in our laboratory and are reviewed in detail below.

BAG3

Mutations in the *BAG3* gene, encoding BCL2-associated athanogene 3 (BAG3), have been found to be causative for isolated adult-onset DCM (572, 807–816) or early-onset skeletal myopathy with or without RCM or HCM (817–819). Moreover, several studies have identified *BAG3* as a genome-wide significant locus for idiopathic DCM (811, 820, 821), all-cause heart failure development (821, 822), or reduced LV ejection fraction after adjusting for preexisting cardiac conditions (823, 824). In particular, the SNP rs2234962, causing a p.C151R

substitution in the BAG3 protein, has been associated with a decreased risk of DCM (811, 816, 820–822). However, why or how the BAG3 C151R allele provides cardioprotection is not known. Several studies have also explored BAG3 as a potential biomarker. In particular, increased BAG3 serum levels have been associated with adverse outcome in heart failure patients (825) and anti-BAG3 antibodies were detected in the serum of patients with chronic heart failure (826). Consistently, decreased BAG3 protein levels have been reported in the heart of patients with end-stage heart failure (815), suggesting that extracellular BAG3 is released by stressed cardiomyocytes, resulting in the production of autoantibodies. BAG3 is a 75-kDa cochaperone belonging to a six-member family of BAG proteins, which share a common conserved COOH-terminal BAG domain that mediates interactions with the HSPA family of chaperones involved in protein quality control (827–830). Furthermore, BAG proteins bind to the antiapoptotic protein BCL2 through their COOH-terminal region (831, 832). In addition to its COOH-terminal BAG domain, BAG3 contains an NH₂-terminal WW domain, binding to proline-rich regions, a PxxP repeat, associated with SH3 domain-containing proteins [e.g., phospholipase C γ , PLC- γ (827) and Src (833)], and two conserved IPV (Ile-Pro-Val) motifs, binding to small HSPs (sHSPs), including HSPB5 (α B-crystallin), HSPB6, and HSPB8 (834–838). In particular, BAG3 physically links ATPase-dependent HSPA family members, which are themselves ATPases, with ATPase-independent sHSPs without enzymatic activity in large multichaperone complexes (836, 838), implicating it in protein folding, chaperone-assisted autophagy to remove misfolded and damaged proteins, inhibition of protein aggregation, and maintenance of mitochondrial stability (reviewed in Refs. 802, 839). Furthermore, BAG3 inhibits apoptosis through its binding to BCL2 (831). BAG3 is strongly expressed in cardiac and skeletal muscle and is localized both in the cytosol and at the Z-line (840), where it interacts with the F-actin capping protein CapZ β 1 (841), thereby maintaining the structural integrity of the Z-line. In particular, BAG3 was found to promote the interaction between CapZ β 1 and HSPA/HSP70, and in the absence of BAG3 CapZ β 1 was lost from the Z-line and degraded by the ubiquitin-proteasome system, resulting in myofibrillar degeneration in response to mechanical stress (841). BAG3 has also been shown to localize to the sarcolemma and t-tubules and coimmunoprecipitate with the β 1-adrenergic receptor and LTCC (842). In-line with this, BAG3 was found to modulate myocyte contractility and excitation-contraction coupling in ventricular cardiomyocytes in response to β -adrenergic stimulation (842). Consistent with a role in cardiac contractility, reduced sarcomeric BAG3 expression was shown to correlate with decreased myofilament contractile function in DCM patients, which was found to be associated with impaired protein turnover, resulting in incorporation of ubiquitinated misfolded sarcomeric proteins in the sarcomere (843).

Global BAG3 KO mice showed impaired postnatal growth and developed DCM, leading to early death within 3–4 wk after birth (840, 844, 845). Cardiomyocyte-specific KO of BAG3 caused progressive DCM, accompanied by reduced levels of sHSPs, suppressed autophagosome formation, and accumulation of insoluble protein aggregates, resulting in premature death (840). It should be pointed out that among the four cardiac sHSPs HSPB5, HSPB6, and HSBP8, but not HSPB7, were

downregulated in cardiomyocyte-specific BAG3 KO mice (835, 840). In agreement with this observation, it has been shown that HSPB5, HSPB6, and HSBP8, but not HSPB7, interact with BAG3, which is critical for their stability, whereas HSPB7 exhibits unique BAG3-independent functions (835, 846–848). Heterozygous cardiac-specific BAG3 KO mice with haploinsufficiency developed progressive systolic dysfunction and LV dilation from 10 wk of age (849). Furthermore, analyses on adult cardiomyocytes showed decreased autophagy flux and increased apoptosis as well as a blunted contractile response to adrenergic stimulation. At 6 wk of age, before the development of LV dysfunction or structural alterations, significantly increased protein ubiquitination and reduced myofibrillar force generation were observed, suggesting that impaired BAG3-mediated turnover of sarcomeric proteins, resulting in integration of ubiquitinated proteins in the sarcomere, is responsible for the contractile dysfunction (843). It was further demonstrated that BAG3 exerts its function via stress-induced chaperone-assisted selective autophagy (CASA) at the Z-line, and several sarcomeric proteins were identified as candidates for BAG3/CASA-mediated turnover. Notably, AAV9-mediated delivery of BAG3 to mice subjected to myocardial infarction, known to be associated with reduced BAG3 levels, restored normal myofibrillar contractile function and sarcomere protein turnover, resulting in improved cardiac systolic function (843), whereas no effect was observed in mice subjected to sham surgery. Thus, BAG3-mediated sarcomere turnover is critical for normal contractile function, and BAG3 may represent a potential therapeutic target. In a recent study, the effect of homozygous and heterozygous cardiomyocyte-specific BAG3 KO in adult mice was studied (850). Under basal conditions, homozygous KO mice showed decreased systolic function, LV dilation, and interstitial fibrosis 4 wk after tamoxifen induction, whereas heterozygous KO mice showed normal cardiac morphology and function. Since BAG3 has been found to be upregulated during physiological and pathological hypertrophic remodeling, homozygous and heterozygous mice were subjected to swimming exercise or chronic phenylephrine infusion to induce cardiac physiological and pathological hypertrophy, respectively. Although both homozygous and heterozygous KO mice showed a blunted hypertrophic response to exercise (physiological hypertrophy), they displayed aggravated pathological maladaptive remodeling in response to phenylephrine treatment. In combination with *in vitro* studies, it was revealed that BAG3 promotes physiological hypertrophy and inhibits pathological remodeling through activation of the AKT/mTOR pathway and inhibition of the Calcineurin/NFATC2 pathway, respectively (850). Thus, several lines of evidence suggest that BAG3 overexpression protects against deterioration of cardiac function under conditions of cardiac stress or injury. It should, however, be noted that transgenic overexpression of BAG3 in the heart caused mildly reduced cardiac systolic function associated with reduced levels of sHSPs due to increased protein turnover via activation of autophagy (851), demonstrating that permanent high-level BAG3 overexpression is not beneficial.

Several KI and Tg mouse models of BAG3 mutations associated with DCM have been studied. A mouse KI model carrying the BAG3 p.E455K mutation, found to be an unequivocal cause of DCM in a large multigenerational family (10), developed

impaired postnatal growth and premature lethality by 4 wk of age, similar to BAG3 global KO mice (840). Cardiomyocyte-specific KI mice for the BAG3 p.E455K mutation largely recapitulated the cardiac phenotype of cardiomyocyte-specific BAG3 KO mice, demonstrating that the BAG3 p.E455K mutation results in loss of function. The BAG3 p.E455K mutation was found to disrupt the binding of BAG3 to HSPA, implying that the interaction is essential for the role of BAG3 in maintaining cardiomyocyte protein homeostasis and cardiac function (840). Several murine models of the BAG3 p.P209L mutation found in patients with severe early-onset skeletal myopathy combined with restrictive cardiomyopathy (817–819) have been reported. A murine KI model carrying the BAG3 p.P215L mutation (equivalent to the BAG3 p.P209L mutation in human) did not display any cardiac abnormalities up to 16 mo of age (852), whereas Tg mice overexpressing the human BAG3 p.P209L mutant either specifically in cardiomyocytes or globally showed distinct pathological phenotypes. Tg mice generated by the use of a cardiomyocyte-specific promoter developed progressive late-onset systolic and diastolic dysfunction associated with cardiomyocyte hypertrophy and increased anterior wall thickness (853), whereas ubiquitous transgenic expression resulted in growth retardation and the development of early-onset restrictive cardiomyopathy, associated with fibrosis, disintegration of sarcomeres, and formation of protein aggregates, recapitulating the human disease in model (854). Notably, the phenotype could be mitigated by AAV-mediated delivery of shRNA against the transgene.

Eukaryotic Elongation Factor 1 Alpha 2

Mutations in eukaryotic elongation factor 1 alpha 2 (eEF1A2), a 50-kDa protein encoded by the *EEF1A2* gene, have been found to cause a neurodevelopmental disorder, in some cases associated with DCM (804–806). eEF1A2 is one of two isoforms of the alpha subunit of the eEF1 complex, which facilitates the GTP-dependent recruitment of aminoacyl-tRNAs to the acceptor site of the ribosomal complex during protein translation (855). Although eEF1A1 is ubiquitously expressed, eEF1A2 expression is restricted to skeletal muscle, heart, brain, and spinal cord (42, 856–858), explaining the clinical manifestations of eEF1A2 mutations. Despite 92% sequence identity between eEF1A1 and eEF1A2, computational models and recent crystal structure have revealed subtle, but significant, differences between the two isoforms, potentially resulting in differences in aminoacyl-tRNA selectivity, actin binding, and oligomerization patterns (859, 860). A distinct pattern of posttranslational modifications, including lipidation, is proposed to be a specific adaptation of eEF1A2, explaining its enrichment in excitable cell types such as neurons and myocytes (860). These subtle changes could provide a structural basis for the diverse functions of eEF1A2 besides delivering aminoacyl-tRNAs to the ribosome, including cell shape regulation via its interaction with phosphatidylinositol-4 kinase III β (861, 862) and carcinogenesis through its interaction with a set of tyrosine kinases and phosphatases via its proline-rich motif (863).

An autosomal recessive mutation in the *EEF1A2* gene, resulting in eEF1A2 ablation, has been shown to be responsible for the spontaneously arising wasted (*Wst*) mutation in an inbred mouse colony (864). Homozygous *Wst* mice

develop profound neuromuscular defects, including muscle wasting, tremors, ataxia, and motor neuron degeneration, starting from ~21 days of age, resulting in death within 28 days after birth (864). This onset of the phenotype coincides with the decline in eEF1A1 expression to undetectable levels by 21 days of age and a concomitant increase in eEF1A2 expression, so that *Wst* mice are deficient for both eEF1A isoforms by 21 days of age (856). Tg expression of eEF1A2 in muscle did not ameliorate the atrophic phenotype of *Wst* mice, indicating that the muscle wasting resulted from the loss of eEF1A2 in neurons (865). Cardiomyocyte-specific KO of eEF1A2 was recently shown to result in the development of rapidly progressing LV dilation and systolic dysfunction from ~6 wk of age, accompanied by cardiac fibrosis, upregulation of markers of cardiac remodeling, and reduced expression of components of the mitochondrial electron transport chain (866). This led to death within 22 wk of age, starting already from 7 wk of age. At the molecular level, compensatory upregulation of the eEF1A1 isoform was observed and no defect in global protein translation was found (866). This suggests that loss of eEF1A2 may only affect the translation of a specific subset of proteins and/or that eEF1A2 may have aminoacyl-tRNA transport-independent functions in the heart. Global KI mice homozygous for the eEF1A2 p.P333L mutation, which has been associated with neurodevelopmental disorder with DCM in patients homozygous for the mutation, showed a phenotype similar to *Wst* mice and died at ~4 wk of age. On the other hand, cardiac-specific eEF1A2-P333L KI mice exhibited essentially the same DCM phenotype as cardiomyocyte-specific eEF1A2 KO mice and showed reduced eEF1A2 protein expression levels, although RNA levels were unaffected, indicating that the mutation constitutes a loss-of-function mutation affecting protein stability.

DISCUSSION

Studies by others and us on sarcomeric and nonsarcomeric proteins, including intermediate filament proteins, SR proteins, costameric proteins, NE proteins, transcription factors, cochaperones, etc., are developing a comprehensive understanding of the complex cytoskeletal and noncytoskeletal network required for cardiac muscle development, structure, and optimal function as well as how mutations in these proteins lead to cardiomyopathies. This advancement in knowledge would not have been possible without genetic mouse models. The CRISPR/Cas9 gene-editing technology has significantly simplified and streamlined the generation of genetic mouse models, and our laboratory has actively used this technology (867). Intensified use of murine models in cardiac research has revealed specific limitations and unexpected advantages as well as stimulating new-lines of future research as discussed below. However, genetic mouse models do not always recapitulate human disease. The work on BAG3 by our laboratory has revealed that the utility of mice as a genetic model not only varies from protein to protein but can even depend on the location and nature of the mutation in the same protein. The BAG3 p.P209L (818, 819, 868) and p.E455K (816) mutations both have a solid clinical indication of their pathogenicity. Still, only BAG3 p.E455K KI recapitulated the human disease, whereas artificial overexpression of the BAG3 p.P209L mutant protein was

required for the mutant to elicit pathology in mice (852–854, 869). Moreover, mouse genetic background can significantly affect the phenotype, implying the existence of powerful modifier genes (870, 871). For example, our group did not observe any overt cardiac phenotype in luma KI and KO models, whereas other groups using different genetic backgrounds partly recapitulated the ACM phenotype observed in humans (727–729). Conversely, differences between phenotypes in a mouse model and human patients might be beneficial and aid translational research. For example, KO of LAMP2, a protein essential for autophagy and responsible for Danon disease, a multisystem disorder characterized by cardiomyopathy (872, 873), results in only mild impairment of cardiac function that does not cause premature death, allowing for the use of LAMP2 KO mice as a preclinical model for testing of potential therapies (874, 875). Similarly, mice can sustain otherwise potentially fatal ventricular tachycardia in humans, making it an ideal model to study and screen for new treatments for arrhythmogenic diseases, such as polymorphic catecholaminergic ventricular arrhythmia (876, 877). However, it should be pointed out that maladaptive responses leading to heart failure in murine hearts differ from those in humans (reviewed in Ref. 878). Therefore, findings from murine studies might not always directly apply to humans. Several drug candidates were shown to be effective in murine models but failed in human trials (reviewed in Ref. 879), indicating species-specific molecular pathways leading to heart failure. Therefore, caution should be taken when pathomechanistically linking genetic defects to the development of cardiomyopathy and heart failure in murine models. As observed in numerous instances in this review, genetic background plays a significant role in whether the murine model will recapitulate the human disease or not. Natural genetic variability of mouse strains can be successfully applied to uncover the genetic bases of cardiovascular pathophysiological manifestations in otherwise hard-to-study diseases, such as nonfamilial forms of heart failure (reviewed in Ref. 880). Moreover, changing genetic background can be a successful strategy to bring a genetic mouse model close to the human disease. For example, the *mdx* mouse model of human dystrophinopathy recapitulates the human disease better in the DBA/2 than the C57BL/10 background (881). Finally, modern genome editing technologies have lifted species limitations and catalyzed the generation and use of nonmurine models for human cardiomyopathies (882, 883).

With their strengths and weaknesses, genetic mouse models will continue to be critical for understanding the pathomechanisms of human cardiomyopathies. The function of many cardiomyopathy-related proteins, such as ALPK3 (884–888), LEMD2 (675, 676), and TAX1BP3 (tax1 binding protein 3) (889) are still elusive, and the molecular mechanisms leading from mutations in certain proteins to cardiomyopathy are not well understood. Furthermore, it is well known that mutations even within the same gene can result in different forms of cardiomyopathy (see Table 1), which may depend on the position of the mutation, affecting different functions of the protein. However, the exact mechanisms still remain elusive. Intriguingly, even the same mutation may lead to distinct forms of cardiomyopathy in different patients, such as the MYPN p.Y20C (159) and MLP p.W4R

(96–99, 102, 108) polymorphisms, which have been associated with both DCM and HCM while being present also in the healthy population. The different outcomes may be explained by the effect of genetic modifier(s) and/or environmental effects. However, future studies on mouse models mimicking distinct human mutations in different mouse strains and in various environmental conditions are needed to determine this. Furthermore, accumulating clinical data have demonstrated the oligogenic nature of specific human cardiomyopathies, and mouse models for these diseases carrying mutations in more than one gene have been proven to be valuable models by several groups, including ours (491, 890–893). Thus, research on oligogenic cardiomyopathies using genetic mouse models will continue to expand. Moreover, despite their clinical relevance, cardiomyopathy-linked mutations in the noncoding regions of the genome remain poorly understood and understudied, opening new research areas (894). Thus, although there has been significant progress in understanding the molecular mechanisms underlying human cardiomyopathies, mouse models will continue to be an essential tool for uncovering still unanswered research questions as well as for testing of potential therapies. Insight into the molecular basis of cardiomyopathy is essential for the identification of new therapeutic strategies specific for each of the distinct forms of cardiomyopathy to be translated to the clinical setting.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.-L.B., J.B., and J.C. prepared figures; M.-L.B. and J.B. drafted manuscript; M.-L.B., J.B., and J.C. edited and revised manuscript; M.-L.B., J.B., and J.C. approved final version of manuscript.

REFERENCES

- Litvinouková M, Talavera-López C, Maatz H, Reichart D, Worth CL, Lindberg EL, et al. Cells of the adult human heart. *Nature* 588: 466–472, 2020. doi:10.1038/s41586-020-2797-4.
- Pinto AR, Ilinykh A, Ivey MJ, Kuwabara JT, D’Antoni ML, Debuque R, Chandran A, Wang L, Arora K, Rosenthal NA, Tallquist MD. Revisiting cardiac cellular composition. *Circ Res* 118: 400–409, 2016. doi:10.1161/CIRCRESAHA.115.307778.
- Gautel M, Djinić-Carugo K. The sarcomeric cytoskeleton: from molecules to motion. *J Exp Biol* 219: 135–145, 2016. doi:10.1242/jeb.124941.
- Huxley AF, Niedergerke R. Structural changes in muscle during contraction. *Nature* 173: 971–973, 1954. doi:10.1038/173971a0.
- Pruna M, Ehler E. The intercalated disc: a mechanosensing signaling node in cardiomyopathy. *Biophys Rev* 12: 931–946, 2020. doi:10.1007/s12551-020-00737-x.
- Sheikh F, Ross RS, Chen J. Cell-cell connection to cardiac disease. *Trends Cardiovasc Med* 19: 182–190, 2009. doi:10.1016/j.tcm.2009.12.001.
- Vermij SH, Abriel H, van Veen TA. Refining the molecular organization of the cardiac intercalated disc. *Cardiovasc Res* 113: 259–275, 2017. doi:10.1093/cvr/cvw259.
- Hong T, Shaw RM. Cardiac t-tubule microanatomy and function. *Physiol Rev* 97: 227–252, 2017. doi:10.1152/physrev.00037.2015.
- Lu F, Pu WT. The architecture and function of cardiac dyads. *Biophys Rev* 12: 1007–1017, 2020. doi:10.1007/s12551-020-00729-x.
- Wei X, Yohannan S, Richards JR. Physiology, cardiac repolarization dispersion and reserve. In: *StatPearls*. Treasure Island, FL: StatPearls Publishing, 2021.
- Piquereau J, Caffin F, Novotova M, Lemaire C, Veksler V, Garnier A, Ventura-Clapier R, Joubert F. Mitochondrial dynamics in the adult cardiomyocytes: which roles for a highly specialized cell? *Front Physiol* 4: 102, 2013. doi:10.3389/fphys.2013.00102.
- Chen J, Chien KR. Complexity in simplicity: monogenic disorders and complex cardiomyopathies. *J Clin Invest* 103: 1483–1485, 1999. doi:10.1172/JCI7297.
- Grimes KM, Prasad V, McNamara JW. Supporting the heart: functions of the cardiomyocyte’s non-sarcomeric cytoskeleton. *J Mol Cell Cardiol* 131: 187–196, 2019. doi:10.1016/j.yjmcc.2019.04.002.
- Elliott P, Andersson B, Arbustini E, Bilinska Z, Cecchi F, Charron P, Dubourg O, Kühn U, Maisch B, McKenna WJ, Monserrat L, Pankuweit S, Rapezzi C, Seferovic P, Tavazzi L, Keren A. Classification of the cardiomyopathies: a position statement from the European Society Of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur Heart J* 29: 270–276, 2008. doi:10.1093/eurheartj/ehm342.
- Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, Moss AJ, Seidman CE, Young JB; American Heart Association, Council on Clinical Cardiology, Heart Failure and Transplantation Committee, Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups, Council on Epidemiology and Prevention. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement 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* 113: 1807–1816, 2006. doi:10.1161/CIRCULATIONAHA.106.174287.
- Elliott P, McKenna WJ. Hypertrophic cardiomyopathy. *Lancet* 363: 1881–1891, 2004. doi:10.1016/S0140-6736(04)16358-7.
- Maron BJ, Gardin JM, Flack JM, Gidding SS, Kurosaki TT, Bild DE. Prevalence of hypertrophic cardiomyopathy in a general population of young adults. Echocardiographic analysis of 4111 subjects in the CARDIA Study. Coronary Artery Risk Development in (Young) Adults. *Circulation* 92: 785–789, 1995. doi:10.1161/01.cir.92.4.785.
- Marian AJ, Braunwald E. Hypertrophic cardiomyopathy: genetics, pathogenesis, clinical manifestations, diagnosis, and therapy. *Circ Res* 121: 749–770, 2017. doi:10.1161/CIRCRESAHA.117.311059.
- Elliott PM, Anastakis A, Borger MA, Borggrefe M, Cecchi F, Charron P, Hagege AA, Lafont A, Limongelli G, Mahrholdt H, McKenna WJ, Mogensen J, Nihoyannopoulos P, Nistri S, Pieper PG, Pieske B, Rapezzi C, Rutten FH, Tillmanns C, Watkins H. 2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy: the Task Force for the Diagnosis and Management of Hypertrophic Cardiomyopathy of the European Society of Cardiology (ESC). *Eur Heart J* 35: 2733–2779, 2014. doi:10.1093/eurheartj/ehu284.
- Marsiglia JD, Pereira AC. Hypertrophic cardiomyopathy: how do mutations lead to disease? *Arq Bras Cardiol* 102: 295–304, 2014. doi:10.5935/abc.20140022.
- Giri P, Mukhopadhyay A, Gupta M, Mohapatra B. Dilated cardiomyopathy: a new insight into the rare but common cause of heart failure. *Heart Fail Rev* (2021). doi:10.1007/s10741-021-10125-6.
- Schultheiss HP, Fairweather D, Caforio AL, Escher F, Hershberger RE, Lipshultz SE, Liu PP, Matsumori A, Mazzanti A, McMurray J, Priori SG. Dilated cardiomyopathy. *Nat Rev Dis Primers* 5: 32, 2019. doi:10.1038/s41572-019-0084-1.

23. McNally EM, Golbus JR, Puckelwartz MJ. Genetic mutations and mechanisms in dilated cardiomyopathy. *J Clin Invest* 123: 19–26, 2013. doi:10.1172/JCI62862.
24. Hershberger RE, Hedges DJ, Morales A. Dilated cardiomyopathy: the complexity of a diverse genetic architecture. *Nat Rev Cardiol* 10: 531–547, 2013. doi:10.1038/nrcardio.2013.105.
25. Michels VV, Moll PP, Miller FA, Tajik AJ, Chu JS, Driscoll DJ, Burnett JC, Rodeheffer RJ, Chesebro JH, Tazelaar HD. The frequency of familial dilated cardiomyopathy in a series of patients with idiopathic dilated cardiomyopathy. *N Engl J Med* 326: 77–82, 1992. doi:10.1056/NEJM199201093260201.
26. Grünig E, Tasman JA, Kücherer H, Franz W, Kübler W, Katus HA. Frequency and phenotypes of familial dilated cardiomyopathy. *J Am Coll Cardiol* 31: 186–194, 1998. doi:10.1016/S0735-1097(97)00434-8.
27. Gregori D, Rocco C, Miodic S, Mestroni L. Estimating the frequency of familial dilated cardiomyopathy in the presence of misclassification errors. *J Appl Stat* 28: 53–62, 2001. doi:10.1080/026647601200011590.
28. Cimiotti D, Budde H, Hassoun R, Jaquet K. Genetic restrictive cardiomyopathy: causes and consequences—an integrative approach. *Int J Mol Sci* 22: 558, 2021. doi:10.3390/ijms22020558.
29. Wittekind SG, Ryan TD, Gao Z, Zafar F, Czosek RJ, Chin CW, Jefferies JL. Contemporary outcomes of pediatric restrictive cardiomyopathy: a single-center experience. *Pediatr Cardiol* 40: 694–704, 2019. doi:10.1007/s00246-018-2043-0.
30. Pereira NL, Grogan M, Dec GW. Spectrum of restrictive and infiltrative cardiomyopathies: part 1 of a 2-part series. *J Am Coll Cardiol* 71: 1130–1148, 2018. doi:10.1016/j.jacc.2018.01.016.
31. Srivastava S, Yavari M, Al-Abcha A, Banga S, Abela G. Ventricular non-compaction review. *Heart Fail Rev*. In press. doi:10.1007/s10741-021-10128-3.
32. Ichida F. Left ventricular noncompaction—risk stratification and genetic consideration. *J Cardiol* 75: 1–9, 2020. doi:10.1016/j.jcc.2019.09.011.
33. Towbin JA, Lorts A, Jefferies JL. Left ventricular non-compaction cardiomyopathy. *Lancet* 386: 813–825, 2015. doi:10.1016/S0140-6736(14)61282-4.
34. Ross SB, Jones K, Blanch B, Puranik R, McGeechan K, Barratt A, Semsarian C. A systematic review and meta-analysis of the prevalence of left ventricular non-compaction in adults. *Eur Heart J* 41: 1428–1436, 2020. doi:10.1093/eurheartj/ehz317.
35. van Waning JI, Caliskan K, Hoedemaekers YM, van Spaendonck-Zwarts KY, Baas AF, Boekholdt SM, van Melle JP, Teske AJ, Asselbergs FW, Backx AP, du Marchie Sarvaas GJ, Dalinghaus M, Breur JM, Linschoten MP, Verlooi LA, Kardys I, Dooijes D, Lekanne Deprez RH, IJpma AS, van den Berg MP, Hofstra RM, van Slegtenhorst MA, Jongbloed JD, Majoor-Krakauer D. Genetics, clinical features, and long-term outcome of noncompaction cardiomyopathy. *J Am Coll Cardiol* 71: 711–722, 2018. doi:10.1016/j.jacc.2017.12.019.
36. Corrado D, Basso C. Arrhythmogenic left ventricular cardiomyopathy. *Heart* 2021: heartjnl-2020-316944, 2021. doi:10.1136/heartjnl-2020-316944.
37. Stevens TL, Wallace MJ, Refaey ME, Roberts JD, Koenig SN, Mohler PJ. Arrhythmogenic cardiomyopathy: molecular insights for improved therapeutic design. *J Cardiovasc Dev Dis* 7: 21, 2020. doi:10.3390/jcdd7020021.
38. van der Voorn SM, Te Riele AS, Basso C, Calkins H, Remme CA, van Veen TA. Arrhythmogenic cardiomyopathy: pathogenesis, pro-arrhythmic remodelling, and novel approaches for risk stratification and therapy. *Cardiovasc Res* 116: 1571–1584, 2020. doi:10.1093/cvr/cvaa084.
39. Sen-Chowdhry S, Syrris P, Prasad SK, Hughes SE, Merrifield R, Ward D, Pennell DJ, McKenna WJ. Left-dominant arrhythmogenic cardiomyopathy: an under-recognized clinical entity. *J Am Coll Cardiol* 52: 2175–2187, 2008. doi:10.1016/j.jacc.2008.09.019.
40. Sen-Chowdhry S, Syrris P, Ward D, Asimaki A, Sevdalis E, McKenna WJ. Clinical and genetic characterization of families with arrhythmogenic right ventricular dysplasia/cardiomyopathy provides novel insights into patterns of disease expression. *Circulation* 115: 1710–1720, 2007. doi:10.1161/CIRCULATIONAHA.106.660241.
41. Ackerman MJ, Priori SG, Willems S, Berul C, Brugada R, Calkins H, Camm AJ, Ellinor PT, Gollub M, Hamilton R, Hershberger RE, Judge DP, Le Marec H, McKenna WJ, Schulze-Bahr E, Semsarian C, Towbin JA, Watkins H, Wilde A, Wolpert C, Zipes DP; Heart Rhythm Society, European Heart Rhythm Association. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies. *Europace* 13: 1077–1109, 2011. doi:10.1093/eurpace/eur245.
42. Basso C, Corrado D, Thiene G. Cardiovascular causes of sudden death in young individuals including athletes. *Cardiol Rev* 7: 127–135, 1999. doi:10.1097/00045415-199905000-00009.
43. Tabib A, Loire R, Chalabreysse L, Meyronnet D, Miras A, Malicier D, Thivolet F, Chevalier P, Bouvagnet P. Circumstances of death and gross and microscopic observations in a series of 200 cases of sudden death associated with arrhythmogenic right ventricular cardiomyopathy and/or dysplasia. *Circulation* 108: 3000–3005, 2003. doi:10.1161/01.CIR.0000108396.65446.21.
44. Alegre-Cebollada J, Kosuri P, Giganti D, Eckels E, Rivas-Pardo JA, Hamdani N, Warren CM, Solaro RJ, Linke WA, Fernandez JM. S-glutathionylation of cryptic cysteines enhances titin elasticity by blocking protein folding. *Cell* 156: 1235–1246, 2014. doi:10.1016/j.cell.2014.01.056.
45. Li H, Linke WA, Oberhauser AF, Carrion-Vazquez M, Kerkvliet JG, Lu H, Marszalek PE, Fernandez JM. Reverse engineering of the giant muscle protein titin. *Nature* 418: 998–1002, 2002. doi:10.1038/nature00938.
46. Chauveau C, Rowell J, Ferreira A. A rising titan: TTN review and mutation update. *Hum Mutat* 35: 1046–1059, 2014. doi:10.1002/humu.22611.
47. Linke WA, Hamdani N. Gigantic business: titin properties and function through thick and thin. *Circ Res* 114: 1052–1068, 2014. doi:10.1161/CIRCRESAHA.114.301286.
48. Towbin JA. Inherited cardiomyopathies. *Circ J* 78: 2347–2356, 2014. doi:10.1253/circj.CJ-14-0893.
49. Bang ML. Animal models of congenital cardiomyopathies associated with mutations in Z-line proteins. *J Cell Physiol* 232: 38–52, 2017. doi:10.1002/jcp.25424.
50. Frank D, Frey N. Cardiac Z-disc signaling network. *J Biol Chem* 286: 9897–9904, 2011. doi:10.1074/jbc.R110.174268.
51. Knöll R, Buyandelger B, Lab M. The sarcomeric Z-disc and Z-discompathies. *J Biomed Biotechnol* 2011: 569628, 2011. doi:10.1155/2011/569628.
52. Sheikh F, Bang ML, Lange S, Chen J. Zeroing in on the role of Cypher in striated muscle function, signaling, and human disease. *Trends Cardiovasc Med* 17: 258–262, 2007. doi:10.1016/j.tcm.2007.09.002.
53. Hernandez DA, Bennett CM, Dunina-Barkovskaya L, Wedig T, Capetanaki Y, Herrmann H, Conover GM. Nebulette is a powerful cytolinker organizing desmin and actin in mouse hearts. *Mol Biol Cell* 27: 3869–3882, 2016. doi:10.1091/mbc.E16-04-0237.
54. Bang ML, Gregorio C, Labelle S. Molecular dissection of the interaction of desmin with the C-terminal region of nebulin. *J Struct Biol* 137: 119–127, 2002. doi:10.1006/jsbi.2002.4457.
55. Jaka O, Casas-Fraile L, Lopez de Munain A, Saenz A. Costamere proteins and their involvement in myopathic processes. *Expert Rev Mol Med* 17: e12, 2015. doi:10.1017/erm.2015.9.
56. Peter AK, Cheng H, Ross RS, Knowlton KU, Chen J. The costamere bridges sarcomeres to the sarcolemma in striated muscle. *Prog Pediatr Cardiol* 31: 83–88, 2011. doi:10.1016/j.ppedcard.2011.02.003.
57. Hastings R, de Villiers CP, Hooper C, Ormondroyd L, Pagnamenta A, Lise S, Salatino S, Knight SJ, Taylor JC, Thomson KL, Arnold L, Chatziefthimiou SD, Konarev PV, Wilmanns M, Ehler E, Ghisleni A, Gautel M, Blair E, Watkins H, Gehrmlich K. Combination of whole genome sequencing, linkage, and functional studies implicates a missense mutation in titin as a cause of autosomal dominant cardiomyopathy with features of left ventricular noncompaction. *Circ Cardiovasc Genet* 9: 426–435, 2016. doi:10.1161/CIRCGENETICS.116.001431.
58. Arimura T, Hayashi T, Terada H, Lee SY, Zhou Q, Takahashi M, Ueda K, Nouchi T, Hohda S, Shibutani M, Hirose M, Chen J, Park JE, Yasunami M, Hayashi H, Kimura A. A Cypher/ZASP mutation associated with dilated cardiomyopathy alters the binding affinity to protein kinase C. *J Biol Chem* 279: 6746–6752, 2004. doi:10.1074/jbc.M311849200.
59. Vatta M, Mohapatra B, Jimenez S, Sanchez X, Faulkner G, Perles Z, Sinagra G, Lin JH, Vu TM, Zhou Q, Bowles KR, Di Lenarda A, Schimmenti L, Fox M, Chrisco MA, Murphy RT, McKenna W, Elliott

- P, Bowles NE, Chen J, Valle G, Towbin JA.** Mutations in Cypher/ZASP in patients with dilated cardiomyopathy and left ventricular non-compaction. *J Am Coll Cardiol* 42: 2014–2027, 2003. doi:10.1016/j.jacc.2003.10.021.
60. **Fratev F, Mihaylova E, Pajeva I.** Combination of genetic screening and molecular dynamics as a useful tool for identification of disease-related mutations: ZASP PDZ domain G54S mutation case. *J Chem Inf Model* 54: 1524–1536, 2014. doi:10.1021/ci5001136.
61. **Theis JL, Bos JM, Bartleson VB, Will ML, Binder J, Vatta M, Towbin JA, Gersh BJ, Ommen SR, Ackerman MJ.** Echocardiographic-determined septal morphology in Z-disc hypertrophic cardiomyopathy. *Biochem Biophys Res Commun* 351: 896–902, 2006. doi:10.1016/j.bbrc.2006.10.119.
62. **Xing Y, Ichida F, Matsuoka T, Isobe T, Ikemoto Y, Higaki T, Tsuji T, Haneda N, Kuwabara A, Chen R, Futatani T, Tsubata S, Watanabe S, Watanabe K, Hirono K, Uese K, Miyawaki T, Bowles KR, Bowles NE, Towbin JA.** Genetic analysis in patients with left ventricular non-compaction and evidence for genetic heterogeneity. *Mol Genet Metab* 88: 71–77, 2006. doi:10.1016/j.ymgme.2005.11.009.
63. **Lopez-Ayala JM, Ortiz-Genga M, Gomez-Milanes I, Lopez-Cuenca D, Ruiz-Espejo F, Sanchez-Munoz JJ, Oliva-Sandoval MJ, Monserrat L, Gimeno JR.** A mutation in the Z-line Cypher/ZASP protein is associated with arrhythmogenic right ventricular cardiomyopathy. *Clin Genet* 88: 172–176, 2015. doi:10.1111/cge.12458.
64. **Zheng J, Chen S, Chen Y, Zhu M, Hong D.** A novel mutation in the PDZ-like motif of ZASP causes distal ZASP-related myofibrillar myopathy. *Neuropathology* 37: 45–51, 2017. doi:10.1111/neup.12328.
65. **Griggs R, Vihola A, Hackman P, Talvinen K, Haravuori H, Faulkner G, Eymard B, Richard I, Selcen D, Engel A, Carpen O, Udd B.** Zaspopathy in a large classic late-onset distal myopathy family. *Brain* 130: 1477–1484, 2007. doi:10.1093/brain/awm006.
66. **Selcen D, Engel AG.** Mutations in myotilin cause myofibrillar myopathy. *Neurology* 62: 1363–1371, 2004. [Erratum in *Neurology* 63: 405, 2004]. doi:10.1212/01.WNL.0000123576.74801.75.
67. **Selcen D, Engel AG.** Mutations in ZASP define a novel form of muscular dystrophy in humans. *Ann Neurol* 57: 269–276, 2005. doi:10.1002/ana.20376.
68. **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* 274: 19807–19813, 1999. doi:10.1074/jbc.274.28.19807.
69. **Faulkner G, Pallavicini A, Formentin E, Comelli A, Ievolella C, Trevisan S, Bortoletto G, Scannapieco P, Salamon M, Mouly V, Valle G, Lanfranchi G.** ZASP: a new Z-band alternatively spliced PDZ-motif protein. *J Cell Biol* 146: 465–475, 1999. doi:10.1083/jcb.146.2.465.
70. **Passier R, Richardson JA, Olson EN.** Oracle, a novel PDZ-LIM domain protein expressed in heart and skeletal muscle. *Mech Dev* 92: 277–284, 2000. doi:10.1016/S0925-4773(99)00330-5.
71. **Huang C, Zhou Q, Liang P, Hollander MS, Sheikh F, Li X, Greaser M, Shelton GD, Evans S, Chen J.** Characterization and in vivo functional analysis of splice variants of cypher. *J Biol Chem* 278: 7360–7365, 2003. doi:10.1074/jbc.M211875200.
72. **Klaavuniemi T, Ylännä J.** Zasp/Cypher internal ZM-motif containing fragments are sufficient to co-localize with alpha-actinin—analysis of patient mutations. *Exp Cell Res* 312: 1299–1311, 2006. doi:10.1016/j.yexcr.2005.12.036.
73. **Martinelli VC, Kyle WB, Kojic S, Vitulo N, Li Z, Belgrano A, Maiuri P, Banks L, Vatta M, Valle G, Faulkner G.** ZASP interacts with the mechanosensing protein Ankrd2 and p53 in the signalling network of striated muscle. *PLoS One* 9: e92259, 2014. doi:10.1371/journal.pone.0092259.
74. **Zhou Q, Chu PH, Huang C, Cheng CF, Martone ME, Knoll G, Shelton GD, Evans S, Chen J.** Ablation of Cypher, a PDZ-LIM domain Z-line protein, causes a severe form of congenital myopathy. *J Cell Biol* 155: 605–612, 2001. doi:10.1083/jcb.200107092.
75. **Frey N, Olson EN.** Calsarcin-3, a novel skeletal muscle-specific member of the calsarcin family, interacts with multiple Z-disc proteins. *J Biol Chem* 277: 13998–14004, 2002. doi:10.1074/jbc.M200712200.
76. **von Nandelstadh P, Ismail M, Gardin C, Suila H, Zara I, Belgrano A, Valle G, Carpen O, Faulkner G.** A class III PDZ binding motif in the myotilin and FATZ families binds enigma family proteins: a common link for Z-disc myopathies. *Mol Cell Biol* 29: 822–834, 2009. doi:10.1128/MCB.01454-08.
77. **Zheng M, Cheng H, Li X, Zhang J, Cui L, Ouyang K, Han L, Zhao T, Gu Y, Dalton ND, Bang ML, Peterson KL, Chen J.** Cardiac-specific ablation of Cypher leads to a severe form of dilated cardiomyopathy with premature death. *Hum Mol Genet* 18: 701–713, 2009. doi:10.1093/hmg/ddn400.
78. **Lin C, Guo X, Lange S, Liu J, Ouyang K, Yin X, Jiang L, Cai Y, Mu Y, Sheikh F, Ye S, Chen J, Ke Y, Cheng H.** Cypher/ZASP is a novel A-kinase anchoring protein. *J Biol Chem* 288: 29403–29413, 2013. doi:10.1074/jbc.M113.470708.
79. **Kuroda S, Tokunaga C, Kiyohara Y, Higuchi O, Konishi H, Mizuno K, Gill GN, Kikkawa U.** Protein-protein interaction of zinc finger LIM domains with protein kinase C. *J Biol Chem* 271: 31029–31032, 1996. doi:10.1074/jbc.271.49.31029.
80. **Yu H, Yuan C, Westenbroek RE, Catterall WA.** The AKAP Cypher/Zasp contributes to β -adrenergic/PKA stimulation of cardiac $Ca_v1.2$ calcium channels. *J Gen Physiol* 150: 883–889, 2018. doi:10.1085/jgp.201711818.
81. **Arimura T, Inagaki N, Hayashi T, Shichi D, Sato A, Hinohara K, Vatta M, Towbin JA, Chikamori T, Yamashina A, Kimura A.** Impaired binding of ZASP/Cypher with phosphoglucomutase 1 is associated with dilated cardiomyopathy. *Cardiovasc Res* 83: 80–88, 2009. doi:10.1093/cvr/cvp119.
82. **Mu Y, Jing R, Peter AK, Lange S, Lin L, Zhang J, Ouyang K, Fang X, Veevers J, Zhou X, Evans SM, Cheng H, Chen J.** Cypher and Enigma homolog protein are essential for cardiac development and embryonic survival. *J Am Heart Assoc* 4: e001950, 2015. doi:10.1161/JAHA.115.001950.
83. **Cheng H, Zheng M, Peter AK, Kimura K, Li X, Ouyang K, Shen T, Cui L, Frank D, Dalton ND, Gu Y, Frey N, Peterson KL, Evans SM, Knowlton KU, Sheikh F, Chen J.** Selective deletion of long but not short Cypher isoforms leads to late-onset dilated cardiomyopathy. *Hum Mol Genet* 20: 1751–1762, 2011. doi:10.1093/hmg/ddr050.
84. **Xuan T, Wang D, Lv J, Pan Z, Fang J, Xiang Y, Cheng H, Wang X, Guo X.** Downregulation of Cypher induces apoptosis in cardiomyocytes via Akt/p38 MAPK signaling pathway. *Int J Med Sci* 17: 2328–2337, 2020. doi:10.7150/ijms.48872.
85. **Li Z, Ai T, Samani K, Xi Y, Tzeng HP, Xie M, Wu S, Ge S, Taylor MD, Dong JW, Cheng J, Ackerman MJ, Kimura A, Sinagra G, Brunelli L, Faulkner G, Vatta M.** A ZASP missense mutation, S196L, leads to cytoskeletal and electrical abnormalities in a mouse model of cardiomyopathy. *Circ Arrhythm Electrophysiol* 3: 646–656, 2010. doi:10.1161/CIRCEP.109.929240.
86. **Xi Y, Ai T, De Lange E, Li Z, Wu G, Brunelli L, Kyle WB, Turker I, Cheng J, Ackerman MJ, Kimura A, Weiss JN, Qu Z, Kim JJ, Faulkner G, Vatta M.** Loss of function of hNav1.5 by a ZASP1 mutation associated with intraventricular conduction disturbances in left ventricular noncompaction. *Circ Arrhythm Electrophysiol* 5: 1017–1026, 2012. doi:10.1161/CIRCEP.111.969220.
87. **Zheng M, Cheng H, Banerjee I, Chen J.** ALP/Enigma PDZ-LIM domain proteins in the heart. *J Mol Cell Biol* 2: 96–102, 2010. doi:10.1093/jmcb/mjp038.
88. **Arola AM, Sanchez X, Murphy RT, Hasle E, Li H, Elliott PM, McKenna WJ, Towbin JA, Bowles NE.** Mutations in PDLIM3 and MYOZ1 encoding myocyte Z-line proteins are infrequently found in idiopathic dilated cardiomyopathy. *Mol Genet Metab* 90: 435–440, 2007. doi:10.1016/j.ymgme.2006.12.008.
89. **Wang D, Fang J, Lv J, Pan Z, Yin X, Cheng H, Guo X.** Novel polymorphisms in PDLIM3 and PDLIM5 gene encoding Z-line proteins increase risk of idiopathic dilated cardiomyopathy. *J Cell Mol Med* 23: 7054–7062, 2019. doi:10.1111/jcmm.14607.
90. **Verdonschot JA, Robinson EL, James KN, Mohamed MW, Claes GR, Casas K, Vanhoutte EK, Hazebroek MR, Kringlen G, Pasierb MR, van den Wijngaard A, Glatz JF, Heymans SR, Krapels IP, Nahas S, Brunner HG, Szklarczyk R.** Mutations in PDLIM5 are rare in dilated cardiomyopathy but are emerging as potential disease modifiers. *Mol Genet Genomic Med* 8: e1049, 2020. doi:10.1002/mgg3.1049.
91. **Cheng H, Kimura K, Peter AK, Cui L, Ouyang K, Shen T, Liu Y, Gu Y, Dalton ND, Evans SM, Knowlton KU, Peterson KL, Chen J.** Loss of enigma homolog protein results in dilated cardiomyopathy. *Circ Res* 107: 348–356, 2010. doi:10.1161/CIRCRESAHA.110.218735.

92. Gregorich ZR, Patel JR, Cai W, Lin Z, Heurer R, Fitzsimons DP, Moss RL, Ge Y. Deletion of Enigma Homologue from the Z-disc slows tension development kinetics in mouse myocardium. *J Gen Physiol* 151: 670–679, 2019. doi:10.1085/jgp.201812214.
93. Pashmforoush M, Pomiès P, Peterson KL, Kubalak S, Ross J Jr, Hefti A, Aebi U, Beckerle MC, Chien KR. Adult mice deficient in actinin-associated LIM-domain protein reveal a developmental pathway for right ventricular cardiomyopathy. *Nat Med* 7: 591–597, 2001. doi:10.1038/87920.
94. Lorenzen-Schmidt I, McCulloch AD, Omens JH. Deficiency of actinin-associated LIM protein alters regional right ventricular function and hypertrophic remodeling. *Ann Biomed Eng* 33: 888–896, 2005. doi:10.1007/s10439-005-3604-y.
95. Hershberger RE, Parks SB, Kushner JD, Li D, Ludwigsen S, Jakobs P, Nauman D, Burgess D, Partain J, Litt M. Coding sequence mutations identified in MYH7, TNNT2, SCN5A, CSRP3, LBD3, and TCAP from 313 patients with familial or idiopathic dilated cardiomyopathy. *Clin Transl Sci* 1: 21–26, 2008. doi:10.1111/j.1752-8062.2008.00017.x.
96. Mohapatra B, Jimenez S, Lin JH, Bowles KR, Coveler KJ, Marx JG, Chrisco MA, Murphy RT, Lurie PR, Schwartz RJ, Elliott PM, Vatta M, McKenna W, Towbin JA, Bowles NE. Mutations in the muscle LIM protein and alpha-actinin-2 genes in dilated cardiomyopathy and endocardial fibroelastosis. *Mol Genet Metab* 80: 207–215, 2003. doi:10.1016/S1096-7192(03)00142-2.
97. Knöll R, Hoshijima M, Hoffman HM, Person V, Lorenzen-Schmidt I, Bang ML, Hayashi T, Shiga N, Yasukawa H, Schaper W, McKenna W, Yokoyama M, Schork NJ, Omens JH, McCulloch AD, Kimura A, Gregorio CC, Poller W, Schaper J, Schultheiss HP, Chien KR. The cardiac mechanical stretch sensor machinery involves a Z disc complex that is defective in a subset of human dilated cardiomyopathy. *Cell* 111: 943–955, 2002. doi:10.1016/S0092-8674(02)01226-6.
98. Bos JM, Poley RN, Ny M, Tester DJ, Xu X, Vatta M, Towbin JA, Gersh BJ, Ommen SR, Ackerman MJ. Genotype-phenotype relationships involving hypertrophic cardiomyopathy-associated mutations in titin, muscle LIM protein, and telethonin. *Mol Genet Metab* 88: 78–85, 2006. doi:10.1016/j.ymgme.2005.10.008.
99. Geier C, Gehmlich K, Ehler E, Hassfeld S, Perrot A, Hayess K, Cardim N, Wenzel K, Erdmann B, Krackhardt F, Posch MG, Osterziel KJ, Bublak A, Nagele H, Scheffold T, Dietz R, Chien KR, Spuler S, Furst DO, Nurnberg P, Ozcelik C. Beyond the sarcomere: CSRP3 mutations cause hypertrophic cardiomyopathy. *Hum Mol Genet* 17: 2753–2765, 2008. [Erratum in *Hum Mol Genet* 17: 3436, 2008].
100. Geier C, Perrot A, Ozcelik C, Binner P, Counsell D, Hoffmann K, Pilz B, Martiniak Y, Gehmlich K, van der Ven PF, Furst DO, Vornwald A, von Hodenberg E, Nurnberg P, Scheffold T, Dietz R, Osterziel KJ. Mutations in the human muscle LIM protein gene in families with hypertrophic cardiomyopathy. *Circulation* 107: 1390–1395, 2003. doi:10.1161/01.CIR.0000056522.82563.5F.
101. Janin A, Bessière F, Chauveau S, Chevalier P, Millat G. First identification of homozygous truncating CSRP3 variants in two unrelated cases with hypertrophic cardiomyopathy. *Gene* 676: 110–116, 2018. doi:10.1016/j.gene.2018.07.036.
102. Newman B, Cescon D, Woo A, Rakowski H, Eriksson MJ, Sole M, Wigle ED, Siminovitch KA. W4R variant in CSRP3 encoding muscle LIM protein in a patient with hypertrophic cardiomyopathy. *Mol Genet Metab* 84: 374–375, 2005. doi:10.1016/j.ymgme.2004.11.013.
103. Salazar-Mendiguchía J, Barriaes-Villa R, Lopes LR, Ochoa JP, Rodríguez-Vilela A, Palomino-Doza J, Larrañaga-Moreira JM, Cicerchia M, Cárdenas-Reyes I, García-Giustiniani D, Brögger N, Fernández G, García S, Santiago L, Vélez P, Ortiz-Genga M, Elliott PM, Monserrat L. The p.(Cys150Tyr) variant in CSRP3 is associated with late-onset hypertrophic cardiomyopathy in heterozygous individuals. *Eur J Med Genet* 63: 104079, 2020. doi:10.1016/j.ejmg.2020.104079.
104. Zolk O, Caroni P, Böhm M. Decreased expression of the cardiac LIM domain protein MLP in chronic human heart failure. *Circulation* 101: 2674–2677, 2000. doi:10.1161/01.CIR.101.23.2674.
105. Vafiadaki E, Arvanitis DA, Sanoudou D. Master regulator of cardiac and skeletal muscle functions. *Gene* 566: 1–7, 2015. doi:10.1016/j.gene.2015.04.077.
106. Arber S, Halder G, Caroni P. Muscle LIM protein, a novel essential regulator of myogenesis, promotes myogenic differentiation. *Cell* 79: 221–231, 1994. doi:10.1016/0092-8674(94)90192-9.
107. Hoffmann C, Moreau F, Moes M, Luthold C, Dieterle M, Goretti E, Neumann K, Steinmetz A, Thomas C. Human muscle LIM protein dimerizes along the actin cytoskeleton and cross-links actin filaments. *Mol Cell Biol* 34: 3053–3065, 2014. doi:10.1128/MCB.00651-14.
108. Knöll R, Kostin S, Klede S, Savvatis K, Klinge L, Stehle I, Gunkel S, Kötter S, Babicz K, Sohns M, Miodic S, Didié M, Knöll G, Zimmermann WH, Thelen P, Bickeböller H, Maier LS, Schaper W, Schaper J, Kraft T, Tschöpe C, Linke WA, Chien KR. A common MLP (muscle LIM protein) variant is associated with cardiomyopathy. *Circ Res* 106: 695–704, 2010. doi:10.1161/CIRCRESAHA.109.206243.
109. Louis HA, Pino JD, Schmeichel KL, Pomiès P, Beckerle MC. Comparison of three members of the cysteine-rich protein family reveals functional conservation and divergent patterns of gene expression. *J Biol Chem* 272: 27484–27491, 1997. doi:10.1074/jbc.272.43.27484.
110. Papalouka V, Arvanitis DA, Vafiadaki E, Mavroidis M, Papadodima SA, Spiliopoulou CA, Kremastinos DT, Kranias EG, Sanoudou D. Muscle LIM protein interacts with cofilin 2 and regulates F-actin dynamics in cardiac and skeletal muscle. *Mol Cell Biol* 29: 6046–6058, 2009. doi:10.1128/MCB.00654-09.
111. Heineke J, Ruetten H, Willenbockel C, Gross SC, Naguib M, Schaefer A, Kempf T, Hilfiker-Kleiner D, Caroni P, Kraft T, Kaiser RA, Molkentin JD, Drexler H, Wollert KC. Attenuation of cardiac remodeling after myocardial infarction by muscle LIM protein-calcineurin signaling at the sarcomeric Z-disc. *Proc Natl Acad Sci USA* 102: 1655–1660, 2005. doi:10.1073/pnas.0405488102.
112. Gupta MP, Samant SA, Smith SH, Shroff SG. HDAC4 and PCAF bind to cardiac sarcomeres and play a role in regulating myofilament contractile activity. *J Biol Chem* 283: 10135–10146, 2008. doi:10.1074/jbc.M710277200.
113. Vafiadaki E, Arvanitis DA, Papalouka V, Terzis G, Roumeliotis TI, Spengos K, Garbis SD, Manta P, Kranias EG, Sanoudou D. Muscle lim protein isoform negatively regulates striated muscle actin dynamics and differentiation. *FEBS J* 281: 3261–3279, 2014. doi:10.1111/febs.12859.
114. Ehler E, Horowitz R, Zuppinger C, Price RL, Perriard E, Leu M, Caroni P, Sussman M, Eppenberger HM, Perriard JC. Alterations at the intercalated disk associated with the absence of muscle LIM protein. *J Cell Biol* 153: 763–772, 2001. doi:10.1083/jcb.153.4.763.
115. Postel R, Vakeel P, Topczewski J, Knöll R, Bakkers J. Zebrafish integrin-linked kinase is required in skeletal muscles for strengthening the integrin-ECM adhesion complex. *Dev Biol* 318: 92–101, 2008. doi:10.1016/j.ydbio.2008.03.024.
116. Flick MJ, Konieczny SF. The muscle regulatory and structural protein MLP is a cytoskeletal binding partner of beta-spectrin. *J Cell Sci* 113: 1553–1564, 2000. doi:10.1242/jcs.113.9.1553.
117. Boateng SY, Senyo SE, Qi L, Goldspink PH, Russell B. Myocyte remodeling in response to hypertrophic stimuli requires nucleocytoplasmic shuttling of muscle LIM protein. *J Mol Cell Cardiol* 47: 426–435, 2009. doi:10.1016/j.jmcc.2009.04.006.
118. Kong Y, Flick MJ, Kudla AJ, Konieczny SF. Muscle LIM protein promotes myogenesis by enhancing the activity of MyoD. *Mol Cell Biol* 17: 4750–4760, 1997. doi:10.1128/MCB.17.8.4750.
119. Boateng SY, Belin RJ, Geenen DL, Margulies KB, Martin JL, Hoshijima M, de Tombe PP, Russell B. Cardiac dysfunction and heart failure are associated with abnormalities in the subcellular distribution and amounts of oligomeric muscle LIM protein. *Am J Physiol Heart Circ Physiol* 292: H259–H269, 2007. doi:10.1152/ajpheart.00766.2006.
120. Arber S, Hunter JJ, Ross J Jr, Hongo M, Sansig G, Borg J, Perriard JC, Chien KR, Caroni P. MLP-deficient mice exhibit a disruption of cardiac cytoarchitectural organization, dilated cardiomyopathy, and heart failure. *Cell* 88: 393–403, 1997. doi:10.1016/S0092-8674(00)81878-4.
121. Su Z, Yao A, Zubair I, Sugishita K, Ritter M, Li F, Hunter JJ, Chien KR, Barry WH. Effects of deletion of muscle LIM protein on myocyte function. *Am J Physiol Heart Circ Physiol* 280: H2665–H2673, 2001. doi:10.1152/ajpheart.2001.280.6.H2665.
122. Wilson AJ, Schoenauer R, Ehler E, Agarkova I, Bennett PM. Cardiomyocyte growth and sarcomerogenesis at the intercalated disc. *Cell Mol Life Sci* 71: 165–181, 2014. doi:10.1007/s00018-013-1374-5.

123. van den Bosch BJ, van den Burg CM, Schoonderwoerd K, Lindsey PJ, Scholte HR, de Coo RF, van Rooij E, Rockman HA, Doevendans PA, Smeets HJ. Regional absence of mitochondria causing energy depletion in the myocardium of muscle LIM protein knockout mice. *Cardiovasc Res* 65: 411–418, 2005. doi:10.1016/j.cardiores.2004.10.025.
124. Esposito G, Santana LF, Dilly K, Cruz JD, Mao L, Lederer WJ, Rockman HA. Cellular and functional defects in a mouse model of heart failure. *Am J Physiol Heart Circ Physiol* 279: H3101–H3112, 2000. doi:10.1152/ajpheart.2000.279.6.H3101.
125. Kemecei P, Miklós Z, Biró T, Marincsák R, Tóth BI, Komlódi-Pasztor E, Barnucz E, Mirk E, Van der Vusse GJ, Ligeti L, Ivanics T. Hearts of surviving MLP-KO mice show transient changes of intracellular calcium handling. *Mol Cell Biochem* 342: 251–260, 2010. doi:10.1007/s11010-010-0492-8.
126. Lorenzen-Schmidt I, Stuyvers BD, ter Keurs HE, Date MO, Hoshijima M, Chien KR, McCulloch AD, Omens JH. Young MLP deficient mice show diastolic dysfunction before the onset of dilated cardiomyopathy. *J Mol Cell Cardiol* 39: 241–250, 2005. doi:10.1016/j.yjmcc.2005.05.006.
127. Omens JH, Usyk TP, Li Z, McCulloch AD. Muscle LIM protein deficiency leads to alterations in passive ventricular mechanics. *Am J Physiol Heart Circ Physiol* 282: H680–H687, 2002. doi:10.1152/ajpheart.00773.2001.
128. Li X, Lu WJ, Li Y, Wu F, Bai R, Ma S, Dong T, Zhang H, Lee AS, Wang Y, Lan F. MLP-deficient human pluripotent stem cell derived cardiomyocytes develop hypertrophic cardiomyopathy and heart failure phenotypes due to abnormal calcium handling. *Cell Death Dis* 10: 610, 2019. doi:10.1038/s41419-019-1826-4.
129. Kuhn C, Frank D, Dierck F, Oehl U, Krebs J, Will R, Lehmann LH, Backs J, Katus HA, Frey N. Cardiac remodeling is not modulated by overexpression of muscle LIM protein (MLP). *Basic Res Cardiol* 107: 262, 2012. doi:10.1007/s00395-012-0262-8.
130. Minamisawa S, Hoshijima M, Chu G, Ward CA, Frank K, Gu Y, Martone ME, Wang Y, Ross J Jr, Kranias EG, Giles WR, Chien KR. Chronic phospholamban-sarcoplasmic reticulum calcium ATPase interaction is the critical calcium cycling defect in dilated cardiomyopathy. *Cell* 99: 313–322, 1999. doi:10.1016/S0092-8674(00)81662-1.
131. Rockman HA, Chien KR, Choi DJ, Iaccarino G, Hunter JJ, Ross J Jr, Lefkowitz RJ, Koch WJ. Expression of a beta-adrenergic receptor kinase 1 inhibitor prevents the development of myocardial failure in gene-targeted mice. *Proc Natl Acad Sci USA* 95: 7000–7005, 1998. doi:10.1073/pnas.95.12.7000.
132. Fajardo G, Zhao M, Urashima T, Farahani S, Hu DQ, Reddy S, Bernstein D. Deletion of the β -adrenergic receptor prevents the development of cardiomyopathy in mice. *J Mol Cell Cardiol* 63: 155–164, 2013. doi:10.1016/j.yjmcc.2013.07.016.
133. Yamamoto R, Akazawa H, Ito K, Toko H, Sano M, Yasuda N, Qin Y, Kudo Y, Sugaya T, Chien KR, Komuro I. Angiotensin II type 1a receptor signals are involved in the progression of heart failure in MLP-deficient mice. *Circ J* 71: 1958–1964, 2007. doi:10.1253/circj.71.1958.
134. Lange S, Gehmlich K, Lun AS, Blondelle J, Hooper C, Dalton ND, Alvarez EA, Zhang X, Bang ML, Abassi YA, Dos Remedios CG, Peterson KL, Chen J, Ehler E. MLP and CARP are linked to chronic PKC α signalling in dilated cardiomyopathy. *Nat Commun* 7: 12120, 2016. doi:10.1038/ncomms12120.
135. Braz JC, Gregory K, Pathak A, Zhao W, Sahin B, Klevitsky R, Kimball TF, Lorenz JN, Nairn AC, Liggett SB, Bodi I, Wang S, Schwartz A, Lakatta EG, DePaoli-Roach AA, Robbins J, Hewett TE, Bibb JA, Westfall MV, Kranias EG, Molkentin JD. PKC- α regulates cardiac contractility and propensity toward heart failure. *Nat Med* 10: 248–254, 2004. doi:10.1038/nm1000.
136. Hambleton M, Hahn H, Plegler ST, Kuhn MC, Klevitsky R, Carr AN, Kimball TF, Hewett TE, Dorn GW 2nd, Koch WJ, Molkentin JD. Pharmacological- and gene therapy-based inhibition of protein kinase Calpha/beta enhances cardiac contractility and attenuates heart failure. *Circulation* 114: 574–582, 2006. doi:10.1161/CIRCULATIONAHA.105.592550.
137. 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* 317: 129–136, 2004. doi:10.1007/s00441-004-0873-y.
138. Purevjav E, Varela J, Morgado M, Kearney DL, Li H, Taylor MD, Arimura T, Moncman CL, McKenna W, Murphy RT, Labeit S, Vatta M, Bowles NE, Kimura A, Boriek AM, Towbin JA. Nebulette mutations are associated with dilated cardiomyopathy and endocardial fibroelastosis. *J Am Coll Cardiol* 56: 1493–1502, 2010. doi:10.1016/j.jacc.2010.05.045.
139. Perrot A, Tomasov P, Villard E, Faludi R, Melacini P, Lossie J, Lohmann N, Richard P, De Bortoli M, Angelini A, Varga-Szemes A, Sperling SR, Simor T, Veselka J, Özcelik C, Charron P. Mutations in NEBL encoding the cardiac Z-disk protein nebulette are associated with various cardiomyopathies. *Arch Med Sci* 12: 263–278, 2016. doi:10.5114/aoms.2016.59250.
140. Arimura T, Nakamura T, Hiroi S, Satoh M, Takahashi M, Ohbuchi N, Ueda K, Nouchi T, Yamaguchi N, Akai J, Matsumori A, Sasayama S, Kimura A. Characterization of the human nebulette gene: a polymorphism in an actin-binding motif is associated with nonfamilial idiopathic dilated cardiomyopathy. *Hum Genet* 107: 440–451, 2000. doi:10.1007/s004390000389.
141. Millevoi S, Trombitas K, Kolmerer B, Kostin S, Schaper J, Pelin K, Granzier H, Labeit S. Characterization of nebulette and nebulin and emerging concepts of their roles for vertebrate Z-discs. *J Mol Biol* 282: 111–123, 1998. doi:10.1006/jmbi.1998.1999.
142. Bang ML, Chen J. Roles of nebulin family members in the heart. *Circ J* 79: 2081–2087, 2015. doi:10.1253/circj.CJ-15-0854.
143. Bang ML, Mudry RE, McElhinny AS, Trombitás K, Geach AJ, Yamasaki R, Sorimachi H, Granzier H, Gregorio CC, Labeit S. Myopalladin, a novel 145-kilodalton sarcomeric protein with multiple roles in Z-disc and I-band protein assemblies. *J Cell Biol* 153: 413–427, 2001. doi:10.1083/jcb.153.2.413.
144. Moncman CL, Wang K. Functional dissection of nebulette demonstrates actin binding of nebulin-like repeats and Z-line targeting of SH3 and linker domains. *Cell Motil Cytoskeleton* 44: 1–22, 1999. doi:10.1002/(SICI)1097-0169(199909)44:1<1::AID-CM1>3.0.CO;2-8.
145. Ma K, Forbes JG, Gutierrez-Cruz G, Wang K. Titin as a giant scaffold for integrating stress and Src homology domain 3-mediated signaling pathways: the clustering of novel overlap ligand motifs in the elastic PEVK segment. *J Biol Chem* 281: 27539–27556, 2006. doi:10.1074/jbc.M604525200.
146. Ma K, Wang K. Interaction of nebulin SH3 domain with titin PEVK and myopalladin: implications for the signaling and assembly role of titin and nebulin. *FEBS Lett* 532: 273–278, 2002. doi:10.1016/S0014-5793(02)03655-4.
147. Witt CC, Burkart C, Labeit D, McNabb M, Wu Y, Granzier H, Labeit S. Nebulin regulates thin filament length, contractility, and Z-disk structure in vivo. *EMBO J* 25: 3843–3855, 2006. doi:10.1038/sj.emboj.7601242.
148. Li B, Zhuang L, Trueb B. Zyxin interacts with the SH3 domains of the cytoskeletal proteins LIM-nebulette and Lasp-1. *J Biol Chem* 279: 20401–20410, 2004. doi:10.1074/jbc.M310304200.
149. Takano K, Watanabe-Takano H, Suetsugu S, Kurita S, Tsujita K, Kimura S, Karatsu T, Takenawa T, Endo T. Nebulin and N-WASP cooperate to cause IGF-1-induced sarcomeric actin filament formation. *Science* 330: 1536–1540, 2010. doi:10.1126/science.1197767.
150. Eulitz S, Sauer F, Pelissier MC, Boisguerin P, Molt S, Schuld J, Orfanos Z, Kley RA, Volkmer R, Wilmanns M, Kirfel G, van der Ven PF, Fürst DO. Identification of Xin-repeat proteins as novel ligands of the SH3 domains of nebulin and nebulette and analysis of their interaction during myofibril formation and remodeling. *Mol Biol Cell* 24: 3215–3226, 2013. doi:10.1091/mbc.e13-04-0202.
151. Holmes WB, Moncman CL. Nebulette interacts with filamin C. *Cell Motil Cytoskeleton* 65: 130–142, 2008. doi:10.1002/cm.20249.
152. Bonzo JR, Norris AA, Esham M, Moncman CL. The nebulette repeat domain is necessary for proper maintenance of tropomyosin with the cardiac sarcomere. *Exp Cell Res* 314: 3519–3530, 2008. doi:10.1016/j.yexcr.2008.09.001.
153. Moncman CL, Wang K. Targeted disruption of nebulette protein expression alters cardiac myofibril assembly and function. *Exp Cell Res* 273: 204–218, 2002. doi:10.1006/excr.2001.5423.
154. Mastrotoato G, Liang X, Li X, Carullo P, Piroddi N, Tesi C, Gu Y, Dalton ND, Peterson KL, Poggesi C, Sheikh F, Chen J, Bang ML. Nebulette knockout mice have normal cardiac function, but show Z-line widening and up-regulation of cardiac stress markers. *Cardiovasc Res* 107: 216–225, 2015. doi:10.1093/cvr/cvv156.

155. **Vejannda RM, Orgil BO, Alberson NR, Li N, Munkhsaikhan U, Khuchua Z, Martherus R, Azeloglu EU, Xu F, Lu L, Towbin JA, Purevjav E.** Deficiency in nebulin repeats of sarcomeric nebulin is detrimental for cardiomyocyte tolerance to exercise and biomechanical stress. *Am J Physiol Heart Circ Physiol* 320: H2130–H2146, 2021. doi:10.1152/ajpheart.00732.2020.
156. **Maiellaro-Rafferty K, Wansapura JP, Mendsaikhan U, Osinska H, James JF, Taylor MD, Robbins J, Kranias EG, Towbin JA, Purevjav E.** Altered regional cardiac wall mechanics are associated with differential cardiomyocyte calcium handling due to nebulin mutations in preclinical inherited dilated cardiomyopathy. *J Mol Cell Cardiol* 60: 151–160, 2013. doi:10.1016/j.jmcc.2013.04.021.
157. **Duboscq-Bidot L, Xu P, Charron P, Neyroud N, Dilanian G, Millaire A, Bors V, Komajda M, Villard E.** Mutations in the Z-band protein myopalladin gene and idiopathic dilated cardiomyopathy. *Cardiovasc Res* 77: 118–125, 2008. doi:10.1093/cvr/cvm015.
158. **Bagnall RD, Yeates L, Semsarian C.** Analysis of the Z-disc genes PDLIM3 and MYPN in patients with hypertrophic cardiomyopathy. *Int J Cardiol* 145: 601–602, 2010. doi:10.1016/j.ijcard.2010.08.004.
159. **Purevjav E, Arimura T, Augustin S, Huby AC, Takagi K, Nunoda S, Kearney DL, Taylor MD, Terasaki F, Bos JM, Ommen SR, Shibata H, Takahashi M, Itoh-Satoh M, McKenna WJ, Murphy RT, Labeit S, Yamanaka Y, Machida N, Park JE, Alexander PM, Weintraub RG, Kitaura Y, Ackerman MJ, Kimura A, Towbin JA.** Molecular basis for clinical heterogeneity in inherited cardiomyopathies due to myopalladin mutations. *Hum Mol Genet* 21: 2039–2053, 2012. doi:10.1093/hmg/dds022.
160. **Meyer T, Ruppert V, Ackermann S, Richter A, Perrot A, Sperling SR, Posch MG, Maisch B, Pankuweit S; German Competence Network Heart Failure.** Novel mutations in the sarcomeric protein myopalladin in patients with dilated cardiomyopathy. *Eur J Hum Genet* 21: 294–300, 2013. doi:10.1038/ejhg.2012.173.
161. **Miyatake S, Mitsuhashi S, Hayashi YK, Purevjav E, Nishikawa A, Koshimizu E, Suzuki M, Yatabe K, Tanaka Y, Ogata K, Kuru S, Shiina M, Tsurusaki Y, Nakashima M, Mizuguchi T, Miyake N, Saito H, Ogata K, Kawai M, Towbin J, Nonaka I, Nishino I, Matsumoto N.** Biallelic mutations in MYPN, encoding myopalladin, are associated with childhood-onset, slowly progressive nemaline myopathy. *Am J Hum Genet* 100: 169–178, 2017. doi:10.1016/j.ajhg.2016.11.017.
162. **Lornage X, Malfatti E, Chéraud C, Schneider R, Biancalana V, Cuisset JM, Garibaldi M, Eymard B, Fardeau M, Boland A, Deleuze JF, Thompson J, Carlier RY, Böhm J, Romero NB, Laporte J.** Recessive MYPN mutations cause cap myopathy with occasional nemaline rods. *Ann Neurol* 81: 467–473, 2017. doi:10.1002/ana.24900.
163. **Merlini L, Sabatelli P, Antoniel M, Carinci V, Niro F, Monetti G, Torella A, Giugliano T, Faldini C, Nigro V.** Congenital myopathy with hanging big toe due to homozygous myopalladin (MYPN) mutation. *Skelet Muscle* 9: 14, 2019. doi:10.1186/s13395-019-0199-9.
164. **Otey CA, Dixon R, Stack C, Goicoechea SM.** Cytoplasmic Ig-domain proteins: cytoskeletal regulators with a role in human disease. *Cell Motil Cytoskeleton* 66: 618–634, 2009. doi:10.1002/cm.20385.
165. **Otey CA, Rachlin A, Moza M, Arneman D, Carpen O.** The palladin/myotilin/myopalladin family of actin-associated scaffolds. *Int Rev Cytol* 246: 31–58, 2005. doi:10.1016/S0074-7696(05)46002-7.
166. **Filomena MC, Yamamoto DL, Carullo P, Medvedev R, Ghisleni A, Piroddi N, Scellini B, Crispino R, D’Autilia F, Zhang J, Felicetta A, Nemska S, Serio S, Tesi C, Catalucci D, Linke WA, Polishchuk R, Poggesi C, Gautel M, Bang ML.** Myopalladin knockout mice develop cardiac dilation and show a maladaptive response to mechanical pressure overload. *Elife* 10: e58313, 2021. doi:10.7554/eLife.58313.
167. **Miller MK, Bang ML, Witt CC, Labeit D, Trombitas C, Watanabe K, Granzier H, McElhinny AS, Gregorio CC, Labeit S.** The muscle ankyrin repeat proteins: CARP, ankrd2/Arpp and DARP as a family of titin filament-based stress response molecules. *J Mol Biol* 333: 951–964, 2003. doi:10.1016/j.jmb.2003.09.012.
168. **Filomena MC, Yamamoto DL, Caremani M, Kadarla VK, Mastrototaro G, Serio S, Vydyanath A, Mutarelli M, Garofalo A, Pertici I, Knöll R, Nigro V, Luther PK, Lieber RL, Beck MR, Linari M, Bang ML.** Myopalladin promotes muscle growth through modulation of the serum response factor pathway. *J Cachexia Sarcopenia Muscle* 11: 169–194, 2020. doi:10.1002/jcsm.12486.
169. **Huby AC, Mendsaikhan U, Takagi K, Martherus R, Wansapura J, Gong N, Osinska H, James JF, Kramer K, Saito K, Robbins J, Khuchua Z, Towbin JA, Purevjav E.** Disturbance in Z-disk mechanosensitive proteins induced by a persistent mutant myopalladin causes familial restrictive cardiomyopathy. *J Am Coll Cardiol* 64: 2765–2776, 2014. doi:10.1016/j.jacc.2014.09.071.
170. **Ono S.** Dynamic regulation of sarcomeric actin filaments in striated muscle. *Cytoskeleton (Hoboken)* 67: 677–692, 2010. doi:10.1002/cm.20476.
171. **Reisler E.** Actin molecular structure and function. *Curr Opin Cell Biol* 5: 41–47, 1993. doi:10.1016/S0955-0674(05)80006-7.
172. **Lehman W, Craig R.** Tropomyosin and the steric mechanism of muscle regulation. *Adv Exp Med Biol* 644: 95–109, 2008. doi:10.1007/978-0-387-85766-4_8.
173. **Kim KH, Pereira NL.** Genetics of cardiomyopathy: clinical and mechanistic implications for heart failure. *Korean Circ J* 51: 797–836, 2021. doi:10.4070/kcj.2021.0154.
174. **Coppini R, Ho CY, Ashley E, Day S, Ferrantini C, Girolami F, Tomberli B, Bardi S, Torricelli F, Cecchi F, Mugelli A, Poggesi C, Tardiff J, Olivetto I.** Clinical phenotype and outcome of hypertrophic cardiomyopathy associated with thin-filament gene mutations. *J Am Coll Cardiol* 64: 2589–2600, 2014. doi:10.1016/j.jacc.2014.09.059.
175. **Frank D, Rangrez AY, Friedrich C, Dittmann S, Stallmeyer B, Yadav P, Bernt A, Schulze-Bahr E, Borlepawar A, Zimmermann WH, Peischard S, Seebohm G, Linke WA, Baba HA, Krüger M, Unger A, Usinger P, Frey N, Schulze-Bahr E.** Cardiac α -actin (ACTC1) gene mutation causes atrial-septal defects associated with late-onset dilated cardiomyopathy. *Circ Genom Precis Med* 12: e002491, 2019. doi:10.1161/CIRCGEN.119.002491.
176. **Gigli M, Begay RL, Morea G, Graw SL, Sinagra G, Taylor MR, Granzier H, Mestroni L.** A review of the giant protein titin in clinical molecular diagnostics of cardiomyopathies. *Front Cardiovasc Med* 3: 21, 2016. doi:10.3389/fcvm.2016.00021.
177. **Knoblauch H, Geier C, Adams S, Budde B, Rudolph A, Zacharias U, Schulz-Menger J, Spuler A, Yaou RB, Nürnberg P, Voit T, Bonne G, Spuler S.** Contractures and hypertrophic cardiomyopathy in a novel FHL1 mutation. *Ann Neurol* 67: 136–140, 2010. doi:10.1002/ana.21839.
178. **Friedrich FW, Wilding BR, Reischmann S, Crocini C, Lang P, Charron P, Müller OJ, McGrath MJ, Vollert I, Hansen A, Linke WA, Hengstenberg C, Bonne G, Morner S, Wichter T, Madeira H, Arbustini E, Eschenhagen T, Mitchell CA, Isnard R, Carrier L.** Evidence for FHL1 as a novel disease gene for isolated hypertrophic cardiomyopathy. *Hum Mol Genet* 21: 3237–3254, 2012. doi:10.1093/hmg/dds157.
179. **Gossios TD, Lopes LR, Elliott PM.** Left ventricular hypertrophy caused by a novel nonsense mutation in FHL1. *Eur J Med Genet* 56: 251–255, 2013. doi:10.1016/j.ejmg.2013.03.001.
180. **Hartmannova H, Kubanek M, Sramko M, Piherova L, Noskova L, Hodanova K, Stranecky V, Pristoupilova A, Sovova J, Marek T, Maluskova J, Ridzon P, Kautzner J, Hulkova H, Kmoch S.** Isolated X-linked hypertrophic cardiomyopathy caused by a novel mutation of the four-and-a-half LIM domain 1 gene. *Circ Cardiovasc Genet* 6: 543–551, 2013. doi:10.1161/CIRCGENETICS.113.000245.
181. **D’Arcy C, Kanellakis V, Forbes R, Wilding B, McGrath M, Howell K, Ryan M, McLean C.** X-linked recessive distal myopathy with hypertrophic cardiomyopathy caused by a novel mutation in the FHL1 gene. *J Child Neurol* 30: 1211–1217, 2015. doi:10.1177/0883073814549807.
182. **Zhang BQ, Si N, Liu DF.** Identification of a novel four and a half LIM domain 1 mutation in a Chinese male presented with hypertrophic cardiomyopathy and mild skeletal muscle hypertrophy. *Chin Med J* 128: 2269–2270, 2015. doi:10.4103/0366-6999.162493.
183. **Gaertner-Rommel A, Tiesmeier J, Jakob T, Strickmann B, Veit G, Bachmann-Mennenga B, Paluszkiwicz L, Klingel K, Schulz U, Laser KT, Karger B, Pfeiffer H, Milting H.** Molecular autopsy and family screening in a young case of sudden cardiac death reveals an unusually severe case of FHL1 related hypertrophic cardiomyopathy. *Mol Genet Genomic Med* 7: e841, 2019.
184. **Giucà A, Mitu C, Popescu BO, Bastian AE, Capşa R, Mursă A, Rădoi V, Popescu BA, Jurcuţ R.** Novel FHL1 mutation variant identified in a patient with nonobstructive hypertrophic cardiomyopathy and myopathy—a case report. *BMC Med Genet* 21: 188, 2020. doi:10.1186/s12881-020-01131-w.

185. San Román I, Navarro M, Martínez F, Albert L, Polo L, Guardiola J, García-Molina E, Muñoz-Esparza C, López-Ayala JM, Sabater-Molina M, Gimeno JR. Unclassified arrhythmic cardiomyopathy associated with Emery-Dreifuss caused by a mutation in FHL1. *Clin Genet* 90: 171–176, 2016. doi:10.1111/cge.12760.
186. Schessl J, Zou Y, McGrath MJ, Cowling BS, Maiti B, Chin SS, Sewry C, Battini R, Hu Y, Cottle DL, Rosenblatt M, Spruce L, Ganguly A, Kirschner J, Judkins AR, Golden JA, Goebel HH, Muntoni F, Flanigan KM, Mitchell CA, Bönnemann CG. Proteomic identification of FHL1 as the protein mutated in human reducing body myopathy. *J Clin Invest* 118: 904–912, 2008. doi:10.1172/JCI34450.
187. Christodoulou DC, Wakimoto H, Onoue K, Eminaga S, Gorham JM, DePalma SR, Herman DS, Teekakirikul P, Conner DA, McKean DM, Domenighetti AA, Aboukhalil A, Chang S, Srivastava G, McDonough B, De Jager PL, Chen J, Bulyk ML, Muehlschlegel JD, Seidman CE, Seidman JG. 5'RNA-Seq identifies Fhl1 as a genetic modifier in cardiomyopathy. *J Clin Invest* 124: 1364–1370, 2014. doi:10.1172/JCI70108.
188. Hwang DM, Dempsey AA, Lee CY, Liew CC. Identification of differentially expressed genes in cardiac hypertrophy by analysis of expressed sequence tags. *Genomics* 66: 1–14, 2000. doi:10.1006/geno.2000.6171.
189. Hwang DM, Dempsey AA, Wang RX, Rezvani M, Barrans JD, Dai KS, Wang HY, Ma H, Cukerman E, Liu YQ, Gu JR, Zhang JH, Tsui SK, Wayne MM, Fung KP, Lee CY, Liew CC. A genome-based resource for molecular cardiovascular medicine: toward a compendium of cardiovascular genes. *Circulation* 96: 4146–4203, 1997. doi:10.1161/01.CIR.96.12.4146.
190. Lim DS, Roberts R, Marian AJ. Expression profiling of cardiac genes in human hypertrophic cardiomyopathy: insight into the pathogenesis of phenotypes. *J Am Coll Cardiol* 38: 1175–1180, 2001. doi:10.1016/S0735-1097(01)01509-1.
191. Chu PH, Ruiz-Lozano P, Zhou Q, Cai C, Chen J. Expression patterns of FHL/SLIM family members suggest important functional roles in skeletal muscle and cardiovascular system. *Mech Dev* 95: 259–265, 2000. doi:10.1016/S0925-4773(00)00341-5.
192. Gaussin V, Tomlinson JE, Depre C, Engelhardt S, Antos CL, Takagi G, Hein L, Topper JN, Liggett SB, Olson EN, Lohse MJ, Vatner SF, Vatner DE. Common genomic response in different mouse models of beta-adrenergic-induced cardiomyopathy. *Circulation* 108: 2926–2933, 2003. doi:10.1161/01.CIR.0000101922.18151.7B.
193. Sheikh F, Raskin A, Chu PH, Lange S, Domenighetti AA, Zheng M, Liang X, Zhang T, Yajima T, Gu Y, Dalton ND, Mahata SK, Dorn GW, Brown JH, Heller-Brown J, Peterson KL, Omens JH, McCulloch AD, Chen J. An FHL1-containing complex within the cardiomyocyte sarcomere mediates hypertrophic biomechanical stress responses in mice. *J Clin Invest* 118: 3870–3880, 2008. [Erratum in *J Clin Invest* 122: 1584, 2012]. doi:10.1172/JCI34472.
194. Friedrich FW, Reischmann S, Schwalm A, Unger A, Ramanujam D, Münch J, Müller OJ, Hengstenberg C, Galve E, Charron P, Linke WA, Engelhardt S, Patten M, Richard P, van der Velden J, Eschenhagen T, Isnard R, Carrier L. FHL2 expression and variants in hypertrophic cardiomyopathy. *Basic Res Cardiol* 109: 451, 2014. doi:10.1007/s00395-014-0451-8.
195. Arimura T, Hayashi T, Matsumoto Y, Shibata H, Hiroi S, Nakamura T, Inagaki N, Hinohara K, Takahashi M, Manatsu SI, Sasaoka T, Izumi T, Bonne G, Schwartz K, Kimura A. Structural analysis of four and half LIM protein-2 in dilated cardiomyopathy. *Biochem Biophys Res Commun* 357: 162–167, 2007. doi:10.1016/j.bbrc.2007.03.128.
196. Lange S, Auerbach D, McLoughlin P, Perriard E, Schäfer BW, Perriard JC, Ehler E. Subcellular targeting of metabolic enzymes to titin in heart muscle may be mediated by DRAL/FHL-2. *J Cell Sci* 115: 4925–4936, 2002. doi:10.1242/jcs.00181.
197. McGrath MJ, Cottle DL, Nguyen MA, Dyson JM, Coghill ID, Robinson PA, Holdsworth M, Cowling BS, Hardeman EC, Mitchell CA, Brown S. Four and a half LIM protein 1 binds myosin-binding protein C and regulates myosin filament formation and sarcomere assembly. *J Biol Chem* 281: 7666–7683, 2006. doi:10.1074/jbc.M512552200.
198. Shathasivam T, Kislinger T, Gramolini AO. Genes, proteins and complexes: the multifaceted nature of FHL family proteins in diverse tissues. *J Cell Mol Med* 14: 2702–2720, 2010. doi:10.1111/j.1582-4934.2010.01176.x.
199. Tran MK, Kurakula K, Koenig DS, de Vries CJ. Protein-protein interactions of the LIM-only protein FHL2 and functional implication of the interactions relevant in cardiovascular disease. *Biochim Biophys Acta* 1863: 219–228, 2016. doi:10.1016/j.bbamcr.2015.11.002.
200. Domenighetti AA, Chu PH, Wu T, Sheikh F, Gokhin DS, Guo LT, Cui Z, Peter AK, Christodoulou DC, Parfenov MG, Gorham JM, Li DY, Banerjee I, Lai X, Witzmann FA, Seidman CE, Seidman JG, Gomes AV, Shelton GD, Lieber RL, Chen J. Loss of FHL1 induces an age-dependent skeletal muscle myopathy associated with myofibrillar and intermyofibrillar disorganization in mice. *Hum Mol Genet* 23: 209–225, 2014. doi:10.1093/hmg/ddt412.
201. D'Angelo DD, Sakata Y, Lorenz JN, Boivin GP, Walsh RA, Liggett SB, Dorn GW 2nd. Transgenic Galphaq overexpression induces cardiac contractile failure in mice. *Proc Natl Acad Sci USA* 94: 8121–8126, 1997. doi:10.1073/pnas.94.15.8121.
202. Raskin A, Lange S, Banares K, Lyon RC, Ziesenis A, Lee LK, Yamazaki KG, Granzier HL, Gregorio CC, McCulloch AD, Omens JH, Sheikh F. A novel mechanism involving four-and-a-half LIM domain protein-1 and extracellular signal-regulated kinase-2 regulates titin phosphorylation and mechanics. *J Biol Chem* 287: 29273–29284, 2012. doi:10.1074/jbc.M112.372839.
203. Geisterfer-Lowrance AA, Christe M, Conner DA, Ingwall JS, Schoen FJ, Seidman CE, Seidman JG. A mouse model of familial hypertrophic cardiomyopathy. *Science* 272: 731–734, 1996. doi:10.1126/science.272.5262.731.
204. Kong Y, Shelton JM, Rothermel B, Li X, Richardson JA, Bassel-Duby R, Williams RS. Cardiac-specific LIM protein FHL2 modifies the hypertrophic response to beta-adrenergic stimulation. *Circulation* 103: 2731–2738, 2001. doi:10.1161/01.CIR.103.22.2731.
205. Kubota A, Juanola-Falgarona M, Emmanuele V, Sanchez-Quintero MJ, Kariya S, Sera F, Homma S, Tanji K, Quinzii CM, Hirano M. Cardiomyopathy and altered integrin-actin signaling in Fhl1 mutant female mice. *Hum Mol Genet* 28: 209–219, 2019. doi:10.1093/hmg/ddy299.
206. Quinzii CM, Vu TH, Min KC, Tanji K, Barral S, Grewal RP, Kattah A, Camaño P, Otaegui D, Kunitatsu T, Blake DM, Wilhelmsen KC, Rowland LP, Hays AP, Bonilla E, Hirano M. X-linked dominant scapuloperoneal myopathy is due to a mutation in the gene encoding four-and-a-half-LIM protein 1. *Am J Hum Genet* 82: 208–213, 2008. doi:10.1016/j.ajhg.2007.09.013.
207. Emmanuele V, Kubota A, Garcia-Diaz B, Garone C, Akman HO, Sánchez-Gutiérrez D, Escudero LM, Kariya S, Homma S, Tanji K, Quinzii CM, Hirano M. Fhl1 W122S causes loss of protein function and late-onset mild myopathy. *Hum Mol Genet* 24: 714–726, 2015. doi:10.1093/hmg/ddu490.
208. Chu PH, Bardwell WM, Gu Y, Ross J Jr, Chen J. FHL2 (SLIM3) is not essential for cardiac development and function. *Mol Cell Biol* 20: 7460–7462, 2000. doi:10.1128/MCB.20.20.7460-7462.2000.
209. Hojaye V, Rothermel BA, Gillette TG, Hill JA. FHL2 binds calcineurin and represses pathological cardiac growth. *Mol Cell Biol* 32: 4025–4034, 2012. doi:10.1128/MCB.05948-11.
210. Purcell NH, Darwis D, Bueno OF, Müller JM, Schüle R, Molkentin JD. Extracellular signal-regulated kinase 2 interacts with and is negatively regulated by the LIM-only protein FHL2 in cardiomyocytes. *Mol Cell Biol* 24: 1081–1095, 2004. doi:10.1128/MCB.24.3.1081-1095.2004.
211. Okamoto R, Li Y, Noma K, Hiroi Y, Liu PY, Taniguchi M, Ito M, Liao JK. FHL2 prevents cardiac hypertrophy in mice with cardiac-specific deletion of ROCK2. *FASEB J* 27: 1439–1449, 2013. doi:10.1096/fj.12-217018.
212. Duboscq-Bidot L, Charron P, Ruppert V, Fauchier L, Richter A, Tavazzi L, Arbustini E, Wichter T, Maisch B, Komajda M, Isnard R, Villard E; EUROGENE Heart Failure Network. Mutations in the ANKRD1 gene encoding CARP are responsible for human dilated cardiomyopathy. *Eur Heart J* 30: 2128–2136, 2009. doi:10.1093/eurheartj/ehp225.
213. Moulik M, Vatta M, Witt SH, Arola AM, Murphy RT, McKenna WJ, Boriek AM, Oka K, Labeit S, Bowles NE, Arimura T, Kimura A, Towbin JA. ANKRD1, the gene encoding cardiac ankyrin repeat protein, is a novel dilated cardiomyopathy gene. *J Am Coll Cardiol* 54: 325–333, 2009. doi:10.1016/j.jacc.2009.02.076.
214. Arimura T, Bos JM, Sato A, Kubo T, Okamoto H, Nishi H, Harada H, Koga Y, Moulik M, Doi YL, Towbin JA, Ackerman MJ, Kimura A. Cardiac ankyrin repeat protein gene (ANKRD1) mutations in

- hypertrophic cardiomyopathy. *J Am Coll Cardiol* 54: 334–342, 2009. doi:10.1016/j.jacc.2008.12.082.
215. Aihara Y, Kurabayashi M, Saito Y, Ohyama Y, Tanaka T, Takeda S, Tomaru K, Sekiguchi K, Arai M, Nakamura T, Nagai R. Cardiac ankyrin repeat protein is a novel marker of cardiac hypertrophy: role of M-CAT element within the promoter. *Hypertension* 36: 48–53, 2000. doi:10.1161/01.hyp.36.1.48.
 216. Bogomolovas J, Brohm K, Cöelutkienė J, Balčiūnaitė G, Bironaitė D, Bukelskienė V, Daunoravičius D, Witt CC, Fielitz J, Grabauskienė V, Labeit S. Induction of Ankrd1 in dilated cardiomyopathy correlates with the heart failure progression. *Biomed Res Int* 2015: 273936, 2015. doi:10.1155/2015/273936.
 217. Herrero I, Roselló-Lletí E, Rivera M, Molina-Navarro MM, Tarazón E, Ortega A, Martínez-Dolz L, Triviño JC, Lago F, González-Juanatey JR, Bertomeu V, Montero JA, Portolés M. RNA-sequencing analysis reveals new alterations in cardiomyocyte cytoskeletal genes in patients with heart failure. *Lab Invest* 94: 645–653, 2014. doi:10.1038/labinvest.2014.54.
 218. Wei YJ, Cui CJ, Huang YX, Zhang XL, Zhang H, Hu SS. Upregulated expression of cardiac ankyrin repeat protein in human failing hearts due to arrhythmogenic right ventricular cardiomyopathy. *Eur J Heart Fail* 11: 559–566, 2009. doi:10.1093/eurjhf/hfp049.
 219. Zolk O, Frohme M, Maurer A, Kluxen FW, Hentsch B, Zubakov D, Hoheisel JD, Zucker IH, Pepe S, Eschenhagen T. Cardiac ankyrin repeat protein, a negative regulator of cardiac gene expression, is augmented in human heart failure. *Biochem Biophys Res Commun* 293: 1377–1382, 2002. doi:10.1016/S0006-291X(02)00387-X.
 220. Badi I, Cinquetti R, Frascoli M, Parolini C, Chiesa G, Taramelli R, Acquati F. Intracellular ANKRD1 protein levels are regulated by 26S proteasome-mediated degradation. *FEBS Lett* 583: 2486–2492, 2009. doi:10.1016/j.febslet.2009.07.001.
 221. Ling SS, Chen YT, Wang J, Richards AM, Liew OW. Ankyrin repeat domain 1 protein: a functionally pleiotropic protein with cardiac biomarker potential. *Int J Mol Sci* 18: 1362, 2017. doi:10.3390/ijms18071362.
 222. Jeyaseelan R, Poizat C, Baker RK, Abdishoo S, Isterabadi LB, Lyons GE, Kedes L. A novel cardiac-restricted target for doxorubicin. CARP, a nuclear modulator of gene expression in cardiac progenitor cells and cardiomyocytes. *J Biol Chem* 272: 22800–22808, 1997. doi:10.1074/jbc.272.36.22800.
 223. Kuo H, Chen J, Ruiz-Lozano P, Zou Y, Nemer M, Chien KR. Control of segmental expression of the cardiac-restricted ankyrin repeat protein gene by distinct regulatory pathways in murine cardiogenesis. *Development* 126: 4223–4234, 1999. doi:10.1242/dev.126.19.4223.
 224. Zou Y, Evans S, Chen J, Kuo HC, Harvey RP, Chien KR. CARP, a cardiac ankyrin repeat protein, is downstream in the Nkx2-5 homeobox gene pathway. *Development* 124: 793–804, 1997. doi:10.1242/dev.124.4.793.
 225. Ihara Y, Suzuki YJ, Kitta K, Jones LR, Ikeda T. Modulation of gene expression in transgenic mouse hearts overexpressing calsequestrin. *Cell Calcium* 32: 21–29, 2002. doi:10.1016/S0143-4160(02)00096-9.
 226. Maeda T, Sepulveda J, Chen HH, Stewart AF. Alpha₁-adrenergic activation of the cardiac ankyrin repeat protein gene in cardiac myocytes. *Gene* 297: 1–9, 2002. doi:10.1016/S0378-1119(02)00924-1.
 227. Torrado M, Lopez E, Centeno A, Castro-Beiras A, Mikhailov AT. Left-right asymmetric ventricular expression of CARP in the piglet heart: regional response to experimental heart failure. *Eur J Heart Fail* 6: 161–172, 2004. doi:10.1016/j.ejheart.2003.11.004.
 228. Zolk O, Marx M, Jackel E, El-Armouche A, Eschenhagen T. Beta-adrenergic stimulation induces cardiac ankyrin repeat protein expression: involvement of protein kinase A and calmodulin-dependent kinase. *Cardiovasc Res* 59: 563–572, 2003. doi:10.1016/S0008-6363(03)00476-0.
 229. Lanzicher T, Zhou T, Saripalli C, Keschrumrus V, Smith Iii JE, Mayans O, Sbaizero O, Granzier H. Single-molecule force spectroscopy on the N2A element of titin: effects of phosphorylation and CARP. *Front Physiol* 11: 173, 2020. doi:10.3389/fphys.2020.00173.
 230. Zhou T, Fleming JR, Franke B, Bogomolovas J, Barsukov I, Rigden DJ, Labeit S, Mayans O. CARP interacts with titin at a unique helical N2A sequence and at the domain Ig81 to form a structured complex. *FEBS Lett* 590: 3098–3110, 2016. doi:10.1002/1873-3468.12362.
 231. Adams M, Fleming JR, Riehle E, Zhou T, Zacharchenko T, Markovic M, Mayans O. Scalable, non-denaturing purification of phosphoproteins using Ga³⁺-IMAC: N2A and M1M2 titin components as study case. *Protein J* 38: 181–189, 2019. doi:10.1007/s10930-019-09815-w.
 232. Lun AS, Chen J, Lange S. Probing muscle ankyrin-repeat protein (MARP) structure and function. *Anat Rec (Hoboken)* 297: 1615–1629, 2014. doi:10.1002/ar.22968.
 233. van der Pijl RJ, van den Berg M, van de Locht M, Shen S, Bogaards SJ, Conijn S, Langlais P, Hooijman PE, Labeit S, Heunks LM, Granzier H, Ottenheijm CA. Muscle ankyrin repeat protein 1 (MARP1) locks titin to the sarcomeric thin filament and is a passive force regulator. *J Gen Physiol* 153: e202112925, 2021. doi:10.1085/jgp.202112925.
 234. Zhou T, Fleming JR, Lange S, Hessel AL, Bogomolovas J, Stronczek C, Grundel D, Ghassemian M, Biju A, Börgesen E, Bullard B, Linke WA, Chen J, Kovermann M, Mayans O. Molecular characterisation of titin N2A and its binding of CARP reveals a titin/actin cross-linking mechanism. *J Mol Biol* 433: 166901, 2021. doi:10.1016/j.jmb.2021.166901.
 235. Laure L, Daniele N, Suel L, Marchand S, Aubert S, Bourg N, Roudaut C, Duguez S, Bartoli M, Richard I. A new pathway encompassing calpain 3 and its newly identified substrate cardiac ankyrin repeat protein is involved in the regulation of the nuclear factor-kappaB pathway in skeletal muscle. *FEBS J* 277: 4322–4337, 2010. doi:10.1111/j.1742-4658.2010.07820.x.
 236. Yura Y, Amano M, Takefuji M, Bando T, Suzuki K, Kato K, Hamaguchi T, Hasanuzzaman Shohag M, Takano T, Funahashi Y, Nakamuta S, Kuroda K, Nishioka T, Murohara T, Kaibuchi K. Focused proteomics revealed a novel Rho-kinase signaling pathway in the heart. *Cell Struct Funct* 41: 105–120, 2016. doi:10.1247/csf.16011.
 237. Witt SH, Labeit D, Granzier H, Labeit S, Witt CC. Dimerization of the cardiac ankyrin protein CARP: implications for MARP titin-based signaling. *J Muscle Res Cell Motil* 26: 401–408, 2005. doi:10.1007/s10974-005-9022-9.
 238. Torrado M, Nespereira B, López E, Centeno A, Castro-Beiras A, Mikhailov AT. ANKRD1 specifically binds CASQ2 in heart extracts and both proteins are co-enriched in piglet cardiac Purkinje cells. *J Mol Cell Cardiol* 38: 353–365, 2005. doi:10.1016/j.yjmcc.2004.11.034.
 239. Witt CC, Witt SH, Lerche S, Labeit D, Back W, Labeit S. Cooperative control of striated muscle mass and metabolism by MuRF1 and MuRF2. *EMBO J* 27: 350–360, 2008. doi:10.1038/sj.emboj.7601952.
 240. Kojic S, Nestorovic A, Rakicevic L, Belgrano A, Stankovic M, Divac A, Faulkner G. A novel role for cardiac ankyrin repeat protein Ankrd1/CARP as a co-activator of the p53 tumor suppressor protein. *Arch Biochem Biophys* 502: 60–67, 2010. doi:10.1016/j.abb.2010.06.029.
 241. Almodóvar-García K, Kwon M, Samaras SE, Davidson JM. ANKRD1 acts as a transcriptional repressor of MMP13 via the AP-1 site. *Mol Cell Biol* 34: 1500–1511, 2014. doi:10.1128/MCB.01357-13.
 242. Lange S, Gehmlich K, Lun AS, Blondelle J, Hooper C, Dalton ND, Alvarez EA, Zhang X, Bang ML, Abassi YA, Dos Remedios CG, Peterson KL, Chen J, Ehler E. MLP and CARP are linked to chronic PKCalpha signalling in dilated cardiomyopathy. *Nat Commun* 7: 12120, 2016. doi:10.1038/ncomms12120.
 243. Chen B, Zhong L, Roush SF, Pentassuglia L, Peng X, Samaras S, Davidson JM, Sawyer DB, Lim CC. Disruption of a GATA4/Ankrd1 signaling axis in cardiomyocytes leads to sarcomere disarray: implications for anthracycline cardiomyopathy. *PLoS One* 7: e35743, 2012. doi:10.1371/journal.pone.0035743.
 244. Zhang N, Ye F, Zhu W, Hu D, Xiao C, Nan J, Su S, Wang Y, Liu M, Gao K, Hu X, Chen J, Yu H, Xie X, Wang J. Cardiac ankyrin repeat protein attenuates cardiomyocyte apoptosis by upregulation of Bcl-2 expression. *Biochim Biophys Acta* 1863: 3040–3049, 2016. doi:10.1016/j.bbamcr.2016.09.024.
 245. Zhong L, Chiusa M, Cadar AG, Lin A, Samaras S, Davidson JM, Lim CC. Targeted inhibition of ANKRD1 disrupts sarcomeric ERK-GATA4 signal transduction and abrogates phenylephrine-induced cardiomyocyte hypertrophy. *Cardiovasc Res* 106: 261–271, 2015. doi:10.1093/cvr/cv108.

246. Liu XH, Bauman WA, Cardozo C. ANKRD1 modulates inflammatory responses in C2C12 myoblasts through feedback inhibition of NF- κ B signaling activity. *Biochem Biophys Res Commun* 464: 208–213, 2015. doi:10.1016/j.bbrc.2015.06.118.
247. Bang ML, Gu Y, Dalton ND, Peterson KL, Chien KR, Chen J. The muscle ankyrin repeat proteins CARP, Ankrd2, and DARP are not essential for normal cardiac development and function at basal conditions and in response to pressure overload. *PLoS One* 9: e93638, 2014. doi:10.1371/journal.pone.0093638.
248. Shen L, Chen C, Wei X, Li X, Luo G, Zhang J, Bin J, Huang X, Cao S, Li G, Liao Y. Overexpression of ankyrin repeat domain 1 enhances cardiomyocyte apoptosis by promoting p53 activation and mitochondrial dysfunction in rodents. *Clin Sci (Lond)* 128: 665–678, 2015. doi:10.1042/CS20140586.
249. Chen C, Shen L, Cao S, Li X, Xuan W, Zhang J, Huang X, Bin J, Xu D, Li G, Kitakaze M, Liao Y. Cytosolic CARP promotes angiotensin II- or pressure overload-induced cardiomyocyte hypertrophy through calcineurin accumulation. *PLoS One* 9: e104040, 2014. doi:10.1371/journal.pone.0104040.
250. Song Y, Xu J, Li Y, Jia C, Ma X, Zhang L, Xie X, Zhang Y, Gao X, Zhang Y, Zhu D. Cardiac ankyrin repeat protein attenuates cardiac hypertrophy by inhibition of ERK1/2 and TGF- β signaling pathways. *PLoS One* 7: e50436, 2012. doi:10.1371/journal.pone.0050436.
251. Piroddi N, Pesce P, Scellini B, Manzini S, Ganzetti GS, Badi I, Menegollo M, Cora V, Tiso S, Cinquetti R, Monti L, Chiesa G, Bleyl SB, Busnelli M, Dellera F, Bruno D, Caicci F, Grimaldi A, Taramelli R, Manni L, Sacerdoti D, Tesi C, Poggese C, Ausoni S, Acquati F, Campione M. Myocardial overexpression of ANKRD1 causes sinus venosus defects and progressive diastolic dysfunction. *Cardiovasc Res* 116: 1458–1472, 2020. doi:10.1093/cvr/cvz291.
252. Cinquetti R, Badi I, Campione M, Bortoletto E, Chiesa G, Parolini C, Camesasca C, Russo A, Taramelli R, Acquati F. Transcriptional deregulation and a missense mutation define ANKRD1 as a candidate gene for total anomalous pulmonary venous return. *Hum Mutat* 29: 468–474, 2008. doi:10.1002/humu.20711.
253. Crocini C, Arimura T, Reischmann S, Eder A, Braren I, Hansen A, Eschenhagen T, Kimura A, Carrier L. Impact of ANKRD1 mutations associated with hypertrophic cardiomyopathy on contraction parameters of engineered heart tissue. *Basic Res Cardiol* 108: 349, 2013. doi:10.1007/s00395-013-0349-x.
254. Mestroni L. Phenotypic heterogeneity of sarcomeric gene mutations: a matter of gain and loss? *J Am Coll Cardiol* 54: 343–345, 2009. doi:10.1016/j.jacc.2009.04.029.
255. Trivedi DV, Adhikari AS, Sarkar SS, Ruppel KM, Spudich JA. Hypertrophic cardiomyopathy and the myosin mesa: viewing an old disease in a new light. *Biophys Rev* 10: 27–48, 2018. doi:10.1007/s12551-017-0274-6.
256. Wang L, Geist J, Grogan A, Hu LR, Kontogianni-Konstantopoulos A. Thick filament protein network, functions, and disease association. *Compr Physiol* 8: 631–709, 2018. doi:10.1002/cphy.c170023.
257. Knöll R. Myosin binding protein C: implications for signal-transduction. *J Muscle Res Cell Motil* 33: 31–42, 2012. doi:10.1007/s10974-011-9281-6.
258. Heling LW, Geeves MA, Kad NM. MyBP-C: one protein to govern them all. *J Muscle Res Cell Motil* 41: 91–101, 2020. doi:10.1007/s10974-019-09567-1.
259. Carrier L, Mearini G, Stathopoulou K, Cuello F. Cardiac myosin-binding protein C (MYBPC3) in cardiac pathophysiology. *Gene* 573: 188–197, 2015. doi:10.1016/j.gene.2015.09.008.
260. Huang W, Szczesna-Cordary D. Molecular mechanisms of cardiomyopathy phenotypes associated with myosin light chain mutations. *J Muscle Res Cell Motil* 36: 433–445, 2015. doi:10.1007/s10974-015-9423-3.
261. Barefield DY, Puckelwartz MJ, Kim EY, Wilsbacher LD, Vo AH, Waters EA, Earley JU, Hadhazy M, Dellefave-Castillo L, Pesce LL, McNally EM. Experimental modeling supports a role for MyBP-HL as a novel myofilament component in arrhythmia and dilated cardiomyopathy. *Circulation* 136: 1477–1491, 2017. doi:10.1161/CIRCULATIONAHA.117.028585.
262. Herman DS, Lam L, Taylor MR, Wang L, Teekakirikul P, Christodoulou D, Conner L, DePalma SR, McDonough B, Sparks E, Teodorescu DL, Cirino AL, Banner NR, Pennell DJ, Graw S, Merlo M, Di Lenarda A, Sinagra G, Bos JM, Ackerman MJ, Mitchell RN, Murry CE, Lakdawala NK, Ho CY, Barton PJ, Cook SA, Mestroni L, Seidman JG, Seidman CE. Truncations of titin causing dilated cardiomyopathy. *N Engl J Med* 366: 619–628, 2012. doi:10.1056/NEJMoa1110186.
263. Franaszczyk M, Chmielewski P, Truszkowska G, Stawinski P, Michalak E, Rydzanicz M, Sobieszczanska-Malek M, Pollak A, Szczygiel J, Kosinska J, Parulski A, Stoklosa T, Tarnowska A, Machnicki MM, Foss-Nieradko B, Szperl M, Sioma A, Kusmierczyk M, Grzybowski J, Zielinski T, Ploski R, Bilinska ZT. Titin truncating variants in dilated cardiomyopathy—prevalence and genotype-phenotype correlations. *PLoS One* 12: e0169007, 2017. doi:10.1371/journal.pone.0169007.
264. Taylor M, Graw S, Sinagra G, Barnes C, Slavov D, Brun F, Pinamonti B, Salcedo EE, Sauer W, Pyxaras S, Anderson B, Simon B, Bogomolovas J, Labeit S, Granzier H, Mestroni L. Genetic variation in titin in arrhythmogenic right ventricular cardiomyopathy-overlap syndromes. *Circulation* 124: 876–885, 2011. doi:10.1161/CIRCULATIONAHA.110.005405.
265. Abraham TP, Jones M, Kazmierczak K, Liang HY, Pinheiro AC, Wagg CS, Lopaschuk GD, Szczesna-Cordary D. Diastolic dysfunction in familial hypertrophic cardiomyopathy transgenic model mice. *Cardiovasc Res* 82: 84–92, 2009. doi:10.1093/cvr/cvp016.
266. Andersen PS, Havndrup O, Bundgaard H, Moolman-Smook JC, Larsen LA, Mogensen J, Brink PA, Børglum AD, Corfield VA, Kjeldsen K, Vuust J, Christiansen M. Myosin light chain mutations in familial hypertrophic cardiomyopathy: phenotypic presentation and frequency in Danish and South African populations. *J Med Genet* 38: E43, 2001. doi:10.1136/jmg.38.12.e43.
267. Andersen PS, Havndrup O, Hougs L, Sørensen KM, Jensen M, Larsen LA, Hedley P, Thomsen AR, Moolman-Smook J, Christiansen M, Bundgaard H. Diagnostic yield, interpretation, and clinical utility of mutation screening of sarcomere encoding genes in Danish hypertrophic cardiomyopathy patients and relatives. *Hum Mutat* 30: 363–370, 2009. doi:10.1002/humu.20862.
268. Chiou KR, Chu CT, Chang MJ. Detection of mutations in symptomatic patients with hypertrophic cardiomyopathy in Taiwan. *J Cardiol* 65: 250–256, 2015. doi:10.1016/j.jjcc.2014.05.010.
269. Claes GR, van Tienen FH, Lindsey P, Kraps IP, Helderma-van den Enden AT, Hoos MB, Barrois YE, Janssen JW, Paulussen AD, Sels JW, Kuijpers SH, van Tintelen JP, van den Berg MP, Heesen WF, Garcia-Pavia P, Perrot A, Christiaans I, Saleminck S, Marcellis CL, Smeets HJ, Brunner HG, Volders PG, van den Wijngaard A. Hypertrophic remodelling in cardiac regulatory myosin light chain (MYL2) founder mutation carriers. *Eur Heart J* 37: 1815–1822, 2016. doi:10.1093/eurheartj/ehv522.
270. Farman GP, Muthu P, Kazmierczak K, Szczesna-Cordary D, Moore JR. Impact of familial hypertrophic cardiomyopathy-linked mutations in the NH₂ terminus of the RLC on β -myosin cross-bridge mechanics. *J Appl Physiol* (1985) 117: 1471–1477, 2014. doi:10.1152/jappphysiol.00798.2014.
271. Flavigny J, Richard P, Isnard R, Carrier L, Charron P, Bonne G, Forissier JF, Desnos M, Dubourg O, Komajda M, Schwartz K, Hainque B. Identification of two novel mutations in the ventricular regulatory myosin light chain gene (MYL2) associated with familial and classical forms of hypertrophic cardiomyopathy. *J Mol Med (Berl)* 76: 208–214, 1998. doi:10.1007/s001090050210.
272. Garcia-Pavia P, Vázquez ME, Segovia J, Salas C, Avellana P, Gómez-Bueno M, Vilches C, Gallardo ME, Garesse R, Molano J, Bornstein B, Alonso-Pulpon L. Genetic basis of end-stage hypertrophic cardiomyopathy. *Eur J Heart Fail* 13: 1193–1201, 2011. doi:10.1093/eurjhf/hfr110.
273. Gil WS, Ávila Vidal LA, Vázquez Salguero MA, Cajiao MB, Peña CV. Genetic variant affecting the myosin light chain 2 related to familial hypertrophic cardiomyopathy. *Intractable Rare Dis Res* 9: 229–232, 2020. doi:10.5582/irdr.2020.03042.
274. Greenberg MJ, Watt JD, Jones M, Kazmierczak K, Szczesna-Cordary D, Moore JR. Regulatory light chain mutations associated with cardiomyopathy affect myosin mechanics and kinetics. *J Mol Cell Cardiol* 46: 108–115, 2009. doi:10.1016/j.yjmcc.2008.09.126.
275. Kabaeva ZT, Perrot A, Wolter B, Dietz R, Cardim N, Correia JM, Schulte HD, Aldashev AA, Mirrahimov MM, Osterziel KJ. Systematic analysis of the regulatory and essential myosin light chain genes: genetic variants and mutations in hypertrophic

- cardiomyopathy. *Eur J Hum Genet* 10: 741–748, 2002. doi:10.1038/sj.ejhg.5200872.
276. **Olivotto I, Girolami F, Ackerman MJ, Nistri S, Bos JM, Zachara E, Ommen SR, Theis JL, Vaubel RA, Re F, Armentano C, Poggesi C, Torricelli F, Cecchi F.** Myofibrillar protein gene mutation screening and outcome of patients with hypertrophic cardiomyopathy. *Mayo Clin Proc* 83: 630–638, 2008. doi:10.4065/83.6.630.
 277. **Poetter K, Jiang H, Hassanzadeh S, Master SR, Chang A, Dalakas MC, Rayment I, Sellers JR, Fananapazir L, Epstein ND.** Mutations in either the essential or regulatory light chains of myosin are associated with a rare myopathy in human heart and skeletal muscle. *Nat Genet* 13: 63–69, 1996. doi:10.1038/ng0596-63.
 278. **Rani DS, Nallari P, Rani J, Nizamuddin S, Seelamneni T, Narasimhan C, Thangaraj K.** A complete absence of missense mutation in myosin regulatory and essential light chain genes of South Indian hypertrophic and dilated cardiomyopathies. *Cardiology* 141: 156–166, 2018. doi:10.1159/000495027.
 279. **Richard P, Charron P, Carrier L, Ledeuil C, Cheav T, Pichereau C, Benaiche A, Isnard R, Dubourg O, Burban M, Gueffet JP, Millaire A, Desnos M, Schwartz K, Hainque B, Komajda M; EUROGENE Heart Failure Project.** Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation* 107: 2227–2232, 2003. [Erratum in *Circulation* 109: 3258, 2004]. doi:10.1161/01.CIR.0000066323.15244.54.
 280. **Santos S, Marques V, Pires M, Silveira L, Oliveira H, Lança V, Brito D, Madeira H, Esteves JF, Freitas A, Carreira IM, Gaspar IM, Monteiro C, Fernandes AR.** High resolution melting: improvements in the genetic diagnosis of hypertrophic cardiomyopathy in a Portuguese cohort. *BMC Med Genet* 13: 17, 2012. doi:10.1186/1471-2350-13-17.
 281. **Szczesna D, Ghosh D, Li Q, Gomes AV, Guzman G, Arana C, Zhi G, Stull JT, Potter JD.** Familial hypertrophic cardiomyopathy mutations in the regulatory light chains of myosin affect their structure, Ca²⁺ binding, and phosphorylation. *J Biol Chem* 276: 7086–7092, 2001. doi:10.1074/jbc.M009823200.
 282. **Huang W, Liang J, Yuan CC, Kazmierczak K, Zhou Z, Morales A, McBride KL, Fitzgerald-Butt SM, Hershberger RE, Szczesna-Cordary D.** Novel familial dilated cardiomyopathy mutation in MYL2 affects the structure and function of myosin regulatory light chain. *FEBS J* 282: 2379–2393, 2015. doi:10.1111/febs.13286.
 283. **Klauke B, Gaertner-Rommel A, Schulz U, Kassner A, Zu Knyphausen E, Laser T, Kececioglu D, Paluszkiwicz L, Blanz U, Sandica E, van den Bogaerd AJ, van Tintelen JP, Gummert J, Milting H.** High proportion of genetic cases in patients with advanced cardiomyopathy including a novel homozygous Plakophilin 2-gene mutation. *PLoS One* 12: e0189489, 2017. doi:10.1371/journal.pone.0189489.
 284. **Weterman MA, Barth PG, van Spaendonck-Zwarts KY, Aronica E, Poil-The BT, Brouwer OF, van Tintelen JP, Qahar Z, Bradley EJ, de Wissel M, Salviati L, Angelini C, van den Heuvel L, Thomasse YE, Backx AP, Nürnberg G, Nürnberg P, Baas F.** Recessive MYL2 mutations cause infantile type I muscle fibre disease and cardiomyopathy. *Brain* 136: 282–293, 2013. doi:10.1093/brain/awt293.
 285. **Marttila M, Win W, Al-Ghamdi F, Abdel-Hamid HZ, Lacomis D, Beggs AH.** MYL2-associated congenital fiber-type disproportion and cardiomyopathy with variants in additional neuromuscular disease genes; the dilemma of panel testing. *Cold Spring Harbor Mol Case Stud* 5: a004184, 2019. doi:10.1101/mcs.a004184.
 286. **Chen J, Kubalak SW, Chien KR.** Ventricular muscle-restricted targeting of the RXRalpha gene reveals a non-cell-autonomous requirement in cardiac chamber morphogenesis. *Development* 125: 1943–1949, 1998. doi:10.1242/dev.125.10.1943.
 287. **Chen J, Kubalak SW, Minamisawa S, Price RL, Becker KD, Hickey R, Ross J Jr, Chien KR.** Selective requirement of myosin light chain 2v in embryonic heart function. *J Biol Chem* 273: 1252–1256, 1998. doi:10.1074/jbc.273.2.1252.
 288. **Warrick HM, Spudich JA.** Myosin structure and function in cell motility. *Annu Rev Cell Biol* 3: 379–421, 1987. doi:10.1146/annurev.cb.03.110187.002115.
 289. **Minamisawa S, Gu Y, Ross J Jr, Chien KR, Chen J.** A post-transcriptional compensatory pathway in heterozygous ventricular myosin light chain 2-deficient mice results in lack of gene dosage effect during normal cardiac growth or hypertrophy. *J Biol Chem* 274: 10066–10070, 1999. doi:10.1074/jbc.274.15.10066.
 290. **Sheikh F, Lyon RC, Chen J.** Getting the skinny on thick filament regulation in cardiac muscle biology and disease. *Trends Cardiovasc Med* 24: 133–141, 2014. doi:10.1016/j.tcm.2013.07.004.
 291. **Sheikh F, Lyon RC, Chen J.** Functions of myosin light chain-2 (MYL2) in cardiac muscle and disease. *Gene* 569: 14–20, 2015. [Erratum in *Gene* 571: 151, 2015]. doi:10.1016/j.gene.2015.06.027.
 292. **Sheikh F, Ouyang K, Campbell SG, Lyon RC, Chuang J, Fitzsimons D, Tangney J, Hidalgo CG, Chung CS, Cheng H, Dalton ND, Gu Y, Kasahara H, Ghassemian M, Omens JH, Peterson KL, Granzier HL, Moss RL, McCulloch AD, Chen J.** Mouse and computational models link Mlc2v dephosphorylation to altered myosin kinetics in early cardiac disease. *J Clin Invest* 122: 1209–1221, 2012. doi:10.1172/JCI61134.
 293. **Ding P, Huang J, Battiprolu PK, Hill JA, Kamm KE, Stull JT.** Cardiac myosin light chain kinase is necessary for myosin regulatory light chain phosphorylation and cardiac performance in vivo. *J Biol Chem* 285: 40819–40829, 2010. doi:10.1074/jbc.M110.160499.
 294. **Warren SA, Briggs LE, Zeng H, Chuang J, Chang EI, Terada R, Li M, Swanson MS, Lecker SH, Willis MS, Spinale FG, Maupin-Furlowe J, McMullen JR, Moss RL, Kasahara H.** Myosin light chain phosphorylation is critical for adaptation to cardiac stress. *Circulation* 126: 2575–2588, 2012. doi:10.1161/CIRCULATIONAHA.112.116202.
 295. **Mizutani H, Okamoto R, Moriki N, Konishi K, Taniguchi M, Fujita S, Dohi K, Onishi K, Suzuki N, Satoh S, Makino N, Itoh T, Hartshorne DJ, Ito M.** Overexpression of myosin phosphatase reduces Ca²⁺ sensitivity of contraction and impairs cardiac function. *Circ J* 74: 120–128, 2010. doi:10.1253/circj.CJ-09-0462.
 296. **Sanbe A, Fewell JG, Gulick J, Osinska H, Lorenz J, Hall DG, Murray LA, Kimball TR, Witt SA, Robbins J.** Abnormal cardiac structure and function in mice expressing nonphosphorylatable cardiac regulatory myosin light chain 2. *J Biol Chem* 274: 21085–21094, 1999. doi:10.1074/jbc.274.30.21085.
 297. **Scruggs SB, Hinken AC, Thawornkaiwong A, Robbins J, Walker LA, de Tombe PP, Geenen DL, Buttrick PM, Solaro RJ.** Ablation of ventricular myosin regulatory light chain phosphorylation in mice causes cardiac dysfunction in situ and affects neighboring myofibrillar protein phosphorylation. *J Biol Chem* 284: 5097–5106, 2009. doi:10.1074/jbc.M807414200.
 298. **Morano I.** Effects of different expression and posttranslational modifications of myosin light chains on contractility of skinned human cardiac fibers. *Basic Res Cardiol* 87: 129–141, 1992.
 299. **Scruggs SB, Reisdorph R, Armstrong ML, Warren CM, Reisdorph N, Solaro RJ, Buttrick PM.** A novel, in-solution separation of endogenous cardiac sarcomeric proteins and identification of distinct charged variants of regulatory light chain. *Mol Cell Proteomics* 9: 1804–1818, 2010. doi:10.1074/mcp.M110.000075.
 300. **van Der Velden J, Klein LJ, Zaremba R, Boontje NM, Huybregts MA, Stoker W, Eijssman L, de Jong JW, Visser CA, Visser FC, Stienen GJ.** Effects of calcium, inorganic phosphate, and pH on isometric force in single skinned cardiomyocytes from donor and failing human hearts. *Circulation* 104: 1140–1146, 2001. doi:10.1161/hc3501.095485.
 301. **van der Velden J, Papp Z, Boontje NM, Zaremba R, de Jong JW, Janssen PM, Hasenfuss G, Stienen GJ.** The effect of myosin light chain 2 dephosphorylation on Ca²⁺-sensitivity of force is enhanced in failing human hearts. *Cardiovasc Res* 57: 505–514, 2003. doi:10.1016/S0008-6363(02)00662-4.
 302. **van der Velden J, Papp Z, Zaremba R, Boontje NM, de Jong JW, Owen VJ, Burton PB, Goldmann P, Jaquet K, Stienen GJ.** Increased Ca²⁺-sensitivity of the contractile apparatus in end-stage human heart failure results from altered phosphorylation of contractile proteins. *Cardiovasc Res* 57: 37–47, 2003. doi:10.1016/S0008-6363(02)00606-5.
 303. **Margossian SS, White HD, Caulfield JB, Norton P, Taylor S, Slayter HS.** Light chain 2 profile and activity of human ventricular myosin during dilated cardiomyopathy. Identification of a causal agent for impaired myocardial function. *Circulation* 85: 1720–1733, 1992. doi:10.1161/01.CIR.85.5.1720.
 304. **Davis JS, Hassanzadeh S, Winitzky S, Lin H, Satorius C, Vemuri R, Aletras AH, Wen H, Epstein ND.** The overall pattern of cardiac contraction depends on a spatial gradient of myosin regulatory light chain phosphorylation. *Cell* 107: 631–641, 2001. doi:10.1016/S0092-8674(01)00586-4.

305. **Szczesna-Cordary D, Guzman G, Zhao J, Hernandez O, Wei J, Diaz-Perez Z.** The E22K mutation of myosin RLC that causes familial hypertrophic cardiomyopathy increases calcium sensitivity of force and ATPase in transgenic mice. *J Cell Sci* 118: 3675–3683, 2005. doi:10.1242/jcs.02492.
306. **Sanbe A, Nelson D, Gulick J, Setser E, Osinska H, Wang X, Hewett TE, Klevitsky R, Hayes E, Warshaw DM, Robbins J.** In vivo analysis of an essential myosin light chain mutation linked to familial hypertrophic cardiomyopathy. *Circ Res* 87: 296–302, 2000. doi:10.1161/01.RES.87.4.296.
307. **Dumka D, Talent J, Akopova I, Guzman G, Szczesna-Cordary D, Borejdo J.** E22K mutation of RLC that causes familial hypertrophic cardiomyopathy in heterozygous mouse myocardium: effect on cross-bridge kinetics. *Am J Physiol Heart Circ Physiol* 291: H2098–H2106, 2006. doi:10.1152/ajpheart.00396.2006.
308. **Szczesna-Cordary D, Jones M, Moore JR, Watt J, Kerrick WG, Xu Y, Wang Y, Wagg C, Lopuschuk GD.** Myosin regulatory light chain E22K mutation results in decreased cardiac intracellular calcium and force transients. *FASEB J* 21: 3974–3985, 2007. doi:10.1096/fj.07-8630.com.
309. **Wang Y, Xu Y, Kerrick WG, Wang Y, Guzman G, Diaz-Perez Z, Szczesna-Cordary D.** Prolonged Ca²⁺ and force transients in myosin RLC transgenic mouse fibers expressing malignant and benign FHC mutations. *J Mol Biol* 361: 286–299, 2006. doi:10.1016/j.jmb.2006.06.018.
310. **Mettikolla P, Calander N, Luchowski R, Gryczynski I, Gryczynski Z, Zhao J, Szczesna-Cordary D, Borejdo J.** Cross-bridge kinetics in myofibrils containing familial hypertrophic cardiomyopathy R58Q mutation in the regulatory light chain of myosin. *J Theor Biol* 284: 71–81, 2011. doi:10.1016/j.jtbi.2011.06.014.
311. **Zaleta-Rivera K, Dainis A, Ribeiro AJ, Cordero P, Rubio G, Shang C, Liu J, Finsterbach T, Parikh VN, Sutton S, Seo K, Sinha N, Jain N, Huang Y, Hajjar RJ, Kay MA, Szczesna-Cordary D, Pruitt BL, Wheeler MT, Ashley EA.** Allele-specific silencing ameliorates restrictive cardiomyopathy attributable to a human myosin regulatory light chain mutation. *Circulation* 140: 765–778, 2019. doi:10.1161/CIRCULATIONAHA.118.036965.
312. **Kerrick WG, Kazmierczak K, Xu Y, Wang Y, Szczesna-Cordary D.** Malignant familial hypertrophic cardiomyopathy D166V mutation in the ventricular myosin regulatory light chain causes profound effects in skinned and intact papillary muscle fibers from transgenic mice. *FASEB J* 23: 855–865, 2009. doi:10.1096/fj.08-118182.
313. **Muthu P, Kazmierczak K, Jones M, Szczesna-Cordary D.** The effect of myosin RLC phosphorylation in normal and cardiomyopathic mouse hearts. *J Cell Mol Med* 16: 911–919, 2012. doi:10.1111/j.1582-4934.2011.01371.x.
314. **Yuan CC, Muthu P, Kazmierczak K, Liang J, Huang W, Irving TC, Kanashiro-Takeuchi RM, Hare JM, Szczesna-Cordary D.** Constitutive phosphorylation of cardiac myosin regulatory light chain prevents development of hypertrophic cardiomyopathy in mice. *Proc Natl Acad Sci USA* 112: E4138–E4146, 2015. doi:10.1073/pnas.1505819112.
315. **Yadav S, Yuan CC, Kazmierczak K, Liang J, Huang W, Takeuchi LM, Kanashiro-Takeuchi RM, Szczesna-Cordary D.** Therapeutic potential of AAV9-S15D-RLC gene delivery in humanized MYL2 mouse model of HCM. *J Mol Med (Berl)* 97: 1033–1047, 2019. doi:10.1007/s00109-019-01791-z.
316. **Huang W, Liang J, Kazmierczak K, Muthu P, Duggal D, Farman GP, Sorensen L, Pozios I, Abraham TP, Moore JR, Borejdo J, Szczesna-Cordary D.** Hypertrophic cardiomyopathy associated Lys104Glu mutation in the myosin regulatory light chain causes diastolic disturbance in mice. *J Mol Cell Cardiol* 74: 318–329, 2014. doi:10.1016/j.yjmcc.2014.06.011.
317. **Huang W, Kazmierczak K, Zhou Z, Aguiar-Pulido V, Narasimhan G, Szczesna-Cordary D.** Gene expression patterns in transgenic mouse models of hypertrophic cardiomyopathy caused by mutations in myosin regulatory light chain. *Arch Biochem Biophys* 601: 121–132, 2016. doi:10.1016/j.abb.2016.02.022.
318. **Yuan CC, Kazmierczak K, Liang J, Zhou Z, Yadav S, Gomes AV, Irving TC, Szczesna-Cordary D.** Sarcomeric perturbations of myosin motors lead to dilated cardiomyopathy in genetically modified MYL2 mice. *Proc Natl Acad Sci USA* 115: E2338–E2347, 2018. doi:10.1073/pnas.1716925115.
319. **Lan F, Lee AS, Liang P, Sanchez-Freire V, Nguyen PK, Wang L, Han L, Yen M, Wang Y, Sun N, Abilez OJ, Hu S, Ebert AD, Navarrete EG, Simmons CS, Wheeler M, Pruitt B, Lewis R, Yamaguchi Y, Ashley EA, Bers DM, Robbins RC, Longaker MT, Wu JC.** Abnormal calcium handling properties underlie familial hypertrophic cardiomyopathy pathology in patient-specific induced pluripotent stem cells. *Cell Stem Cell* 12: 101–113, 2013. doi:10.1016/j.stem.2012.10.010.
320. **Zhou W, Bos JM, Ye D, Tester DJ, Hrstka S, Maleszewski JJ, Ommen SR, Nishimura RA, Schaff HV, Kim CS, Ackerman MJ.** Induced pluripotent stem cell-derived cardiomyocytes from a patient with MYL2-R58Q-mediated apical hypertrophic cardiomyopathy show hypertrophy, myofibrillar disarray, and calcium perturbations. *J Cardiovasc Transl Res* 12: 394–403, 2019. doi:10.1007/s12265-019-09873-6.
321. **Lange S, Pinotsis N, Agarkova I, Ehler E.** The M-band: the underestimated part of the sarcomere. *Biochim Biophys Acta Mol Cell Res* 1867: 118440, 2020. doi:10.1016/j.bbamcr.2019.02.003.
322. **Agarkova IE, Ehler E.** The M-band: not just inert glue but playing an active role in the middle of the sarcomere. In: *Cardiac Cytoarchitecture*, edited by Ehler E. Cham, Switzerland: Springer, 2015.
323. **Carmignac V, Salih MA, Quijano-Roy S, Marchand S, Al Rayess MM, Mukhtar MM, Urtizberea JA, Labeit S, Guicheney P, Leturcq F, Gautel M, Fardeau M, Campbell KP, Richard I, Estournet B, Ferreiro A.** C-terminal titin deletions cause a novel early-onset myopathy with fatal cardiomyopathy. *Ann Neurol* 61: 340–351, 2007. doi:10.1002/ana.21089.
324. **Chauveau C, Bonnemann CG, Julien C, Kho AL, Marks H, Talim B, Maury P, Arne-Bes MC, Uro-Coste E, Alexandrovich A, Vihola A, Schafer S, Kaufmann B, Medne L, Hübner N, Foley AR, Santi M, Udd B, Topaloglu H, Moore SA, Gotthardt M, Samuels ME, Gautel M, Ferreiro A.** Recessive TTN truncating mutations define novel forms of core myopathy with heart disease. *Hum Mol Genet* 23: 980–991, 2014. doi:10.1093/hmg/ddt494.
325. **Siegert R, Perrot A, Keller S, Behlke J, Michalewska-Włodarczyk A, Wycisk A, Tondera M, Morano I, Ozcelik C.** A myomesin mutation associated with hypertrophic cardiomyopathy deteriorates dimerisation properties. *Biochem Biophys Res Commun* 405: 473–479, 2011. doi:10.1016/j.bbrc.2011.01.056.
326. **Shakeel M, Ifran M, Khan IA.** Rare genetic mutations in Pakistani patients with dilated cardiomyopathy. *Gene* 673: 134–139, 2018. doi:10.1016/j.gene.2018.06.019.
327. **Schoenauer R, Emmert MY, Felley A, Ehler E, Brokopp C, Weber B, Nemir M, Faggian GG, Pedrazzini T, Falk V, Hoerstrup SP, Agarkova I.** EH-myomesin splice isoform is a novel marker for dilated cardiomyopathy. *Basic Res Cardiol* 106: 233–247, 2011. doi:10.1007/s00395-010-0131-2.
328. **Arimura T, Matsumoto Y, Okazaki O, Hayashi T, Takahashi M, Inagaki N, Hinohara K, Ashizawa N, Yano K, Kimura A.** Structural analysis of obscurin gene in hypertrophic cardiomyopathy. *Biochem Biophys Res Commun* 362: 281–287, 2007. doi:10.1016/j.bbrc.2007.07.183.
329. **Xu J, Li Z, Ren X, Dong M, Li J, Shi X, Zhang Y, Xie W, Sun Z, Liu X, Dai Q.** Investigation of pathogenic genes in Chinese sporadic hypertrophic cardiomyopathy patients by whole exome sequencing. *Sci Rep* 5: 16609, 2015. doi:10.1038/srep16609.
330. **Marston S, Montgiraud C, Munster AB, Copeland O, Choi O, Dos Remedios C, Messer AE, Ehler E, Knöll R.** OBSCN mutations associated with dilated cardiomyopathy and haploinsufficiency. *PLoS One* 10: e0138568, 2015. doi:10.1371/journal.pone.0138568.
331. **Rowland TJ, Graw SL, Sweet ME, Gigli M, Taylor MR, Mestroni L.** Obscurin variants in patients with left ventricular noncompaction. *J Am Coll Cardiol* 68: 2237–2238, 2016. doi:10.1016/j.jacc.2016.08.052.
332. **Chen P, Xiao Y, Wang Y, Zheng Z, Chen L, Yang X, Li J, Wu W, Zhang S.** Intracellular calcium current disorder and disease phenotype in OBSCN mutant iPSC-based cardiomyocytes in arrhythmogenic right ventricular cardiomyopathy. *Theranostics* 10: 11215–11229, 2020. doi:10.7150/tno.45172.
333. **Makarenko I, Opitz CA, Leake MC, Neagoe C, Kulke M, Gwathmey JK, del Monte F, Hajjar RJ, Linke WA.** Passive stiffness changes caused by upregulation of compliant titin isoforms in human dilated cardiomyopathy hearts. *Circ Res* 95: 708–716, 2004. doi:10.1161/01.RES.0000143901.37063.2f.

334. Wu Y, Bell SP, Trombitas K, Witt CC, Labeit S, LeWinter MM, Granzier H. Changes in titin isoform expression in pacing-induced cardiac failure give rise to increased passive muscle stiffness. *Circulation* 106: 1384–1389, 2002. doi:10.1161/01.CIR.0000029804.61510.02.
335. Borisov AB, Raeker MO, Kontrogianni-Konstantopoulos A, Yang K, Kurnit DM, Bloch RJ, Russell MW. Rapid response of cardiac obscurin gene cluster to aortic stenosis: differential activation of Rho-GEF and MLCK and involvement in hypertrophic growth. *Biochem Biophys Res Commun* 310: 910–918, 2003. doi:10.1016/j.bbrc.2003.09.035.
336. Russell MW, Raeker MO, Korytkowski KA, Sonneman KJ. Identification, tissue expression and chromosomal localization of human Obscurin-MLCK, a member of the titin and Dbl families of myosin light chain kinases. *Gene* 282: 237–246, 2002. doi:10.1016/S0378-1119(01)00795-8.
337. Sutter SB, Raeker MO, Borisov AB, Russell MW. Orthologous relationship of obscurin and Unc-89: phylogeny of a novel family of tandem myosin light chain kinases. *Dev Genes Evol* 214: 352–359, 2004.
338. Young P, Ehler E, Gautel M. Obscurin, a giant sarcomeric Rho guanine nucleotide exchange factor protein involved in sarcomere assembly. *J Cell Biol* 154: 123–136, 2001. doi:10.1083/jcb.200102110.
339. Fukuzawa A, Idowu S, Gautel M. Complete human gene structure of obscurin: implications for isoform generation by differential splicing. *J Muscle Res Cell Motil* 26: 427–434, 2005. doi:10.1007/s10974-005-9025-6.
340. Bowman AL, Kontrogianni-Konstantopoulos A, Hirsch SS, Geisler SB, Gonzalez-Serratos H, Russell MW, Bloch RJ. Different obscurin isoforms localize to distinct sites at sarcomeres. *FEBS Lett* 581: 1549–1554, 2007. doi:10.1016/j.febslet.2007.03.011.
341. Ackermann MA, King B, Lieberman NA, Bobbili PJ, Rudloff M, Berndsen CE, Wright NT, Hecker PA, Kontrogianni-Konstantopoulos A. Novel obscurins mediate cardiomyocyte adhesion and size via the PI3K/AKT/mTOR signaling pathway. *J Mol Cell Cardiol* 111: 27–39, 2017. doi:10.1016/j.yjmcc.2017.08.004.
342. Manning HR, Carter OA, Ackermann MA. Obscure functions: the location-function relationship of obscurins. *Biophys Rev* 9: 245–258, 2017. doi:10.1007/s12551-017-0254-x.
343. Fukuzawa A, Lange S, Holt M, Vihola A, Carmignac V, Ferreira A, Udd B, Gautel M. Interactions with titin and myomesin target obscurin and obscurin-like 1 to the M-band: implications for hereditary myopathies. *J Cell Sci* 121: 1841–1851, 2008. doi:10.1242/jcs.028019.
344. Ford-Speelman DL, Roche JA, Bowman AL, Bloch RJ. The rho-guanine nucleotide exchange factor domain of obscurin activates rhoA signaling in skeletal muscle. *Mol Biol Cell* 20: 3905–3917, 2009. doi:10.1091/mbc.e08-10-1029.
345. Ackermann MA, Hu LY, Bowman AL, Bloch RJ, Kontrogianni-Konstantopoulos A. Obscurin interacts with a novel isoform of MyBP-C slow at the periphery of the sarcomeric M-band and regulates thick filament assembly. *Mol Biol Cell* 20: 2963–2978, 2009. doi:10.1091/mbc.e08-12-1251.
346. Bang ML, Centner T, Fornoff F, Geach AJ, Gotthardt M, McNabb M, Witt CC, Labeit D, Gregorio CC, Granzier H, Labeit S. The complete gene sequence of titin, expression of an unusual approximately 700-kDa titin isoform, and its interaction with obscurin identify a novel Z-line to I-band linking system. *Circ Res* 89: 1065–1072, 2001. doi:10.1161/hh2301.100981.
347. Bowman AL, Catino DH, Strong JC, Randall WR, Kontrogianni-Konstantopoulos A, Bloch RJ. The rho-guanine nucleotide exchange factor domain of obscurin regulates assembly of titin at the Z-disk through interactions with Ran binding protein 9. *Mol Biol Cell* 19: 3782–3792, 2008. doi:10.1091/mbc.e08-03-0237.
348. Kontrogianni-Konstantopoulos A, Jones EM, Van Rossum DB, Bloch RJ. Obscurin is a ligand for small ankyrin 1 in skeletal muscle. *Mol Biol Cell* 14: 1138–1148, 2003. doi:10.1091/mbc.e02-07-0411.
349. Randazzo D, Giacomello E, Lorenzini S, Rossi D, Pierantozzi E, Blaauw B, Reggiani C, Lange S, Peter AK, Chen J, Sorrentino V. Obscurin is required for ankyrinB-dependent dystrophin localization and sarcolemma integrity. *J Cell Biol* 200: 523–536, 2013. doi:10.1083/jcb.201205118.
350. Cunha SR, Mohler PJ. Obscurin targets ankyrin-B and protein phosphatase 2A to the cardiac M-line. *J Biol Chem* 283: 31968–31980, 2008. doi:10.1074/jbc.M806050200.
351. Bagnato P, Barone V, Giacomello E, Rossi D, Sorrentino V. Binding of an ankyrin-1 isoform to obscurin suggests a molecular link between the sarcoplasmic reticulum and myofibrils in striated muscles. *J Cell Biol* 160: 245–253, 2003. doi:10.1083/jcb.200208109.
352. Hu LY, Kontrogianni-Konstantopoulos A. The kinase domains of obscurin interact with intercellular adhesion proteins. *FASEB J* 27: 2001–2012, 2013. doi:10.1096/fj.12-221317.
353. Randazzo D, Pierantozzi E, Rossi D, Sorrentino V. The potential of obscurin as a therapeutic target in muscle disorders. *Expert Opin Ther Targets* 21: 897–910, 2017. doi:10.1080/14728222.2017.1361931.
354. Lange S, Ouyang K, Meyer G, Cui L, Cheng H, Lieber RL, Chen J. Obscurin determines the architecture of the longitudinal sarcoplasmic reticulum. *J Cell Sci* 122: 2640–2650, 2009. doi:10.1242/jcs.046193.
355. Lange S, Perera S, Teh P, Chen J. Obscurin and KCTD6 regulate cullin-dependent small ankyrin-1 (sAnk1.5) protein turnover. *Mol Biol Cell* 23: 2490–2504, 2012. doi:10.1091/mbc.e12-01-0052.
356. Randazzo D, Blaauw B, Paolini C, Pierantozzi E, Spinozzi S, Lange S, Chen J, Protasi F, Reggiani C, Sorrentino V. Exercise-induced alterations and loss of sarcomeric M-line organization in the diaphragm muscle of obscurin knockout mice. *Am J Physiol Cell Physiol* 312: C16–C28, 2017. doi:10.1152/ajpcell.00098.2016.
357. Blondelle J, Marocco V, Clark M, Desmond P, Myers S, Nguyen J, Wright M, Bremner S, Pierantozzi E, Ward S, Estève E, Sorrentino V, Ghassemian M, Lange S. Murine obscurin and Obsl1 have functionally redundant roles in sarcolemmal integrity, sarcoplasmic reticulum organization, and muscle metabolism. *Commun Biol* 2: 178, 2019. doi:10.1038/s42003-019-0405-7.
358. Hu LR, Ackermann MA, Hecker PA, Prosser BL, King B, O'Connell KA, Grogan A, Meyer LC, Berndsen CE, Wright NT, Jonathan Lederer W, Kontrogianni-Konstantopoulos A. Deregulated Ca²⁺ cycling underlies the development of arrhythmia and heart disease due to mutant obscurin. *Sci Adv* 3: e1603081, 2017. doi:10.1126/sciadv.1603081.
359. Fukuzawa A, Koch D, Grover S, Rees M, Gautel M. When is an obscurin variant pathogenic? The impact of Arg4344Gln and Arg4444Trp variants on protein-protein interactions and protein stability. *Hum Mol Genet* 30: 1131–1141, 2021. doi:10.1093/hmg/ddab010.
360. Manrai AK, Funke BH, Rehm HL, Olesen MS, Maron BA, Szolovits P, Margulies DM, Loscalzo J, Kohane IS. Genetic misdiagnoses and the potential for health disparities. *N Engl J Med* 375: 655–665, 2016. doi:10.1056/NEJMsa1507092.
361. Grogan A, Coleman A, Joca H, Granzier H, Russel MW, Ward CW, Kontrogianni-Konstantopoulos A. Deletion of obscurin immunoglobulin domains Ig58/59 leads to age-dependent cardiac remodeling and arrhythmia. *Basic Res Cardiol* 115: 60, 2020. doi:10.1007/s00395-020-00818-8.
362. Pigors M, Schwiager-Briel A, Cosgarea R, Diaconeasa A, Bruckner-Tuderman L, Fleck T, Has C. Desmoplakin mutations with palmoplantar keratoderma, woolly hair and cardiomyopathy. *Acta Derm Venereol* 95: 337–340, 2015. doi:10.2340/00015555-1974.
363. Tester DJ, Ackerman JP, Giudicessi JR, Ackerman NC, Cerrone M, Delmar M, Ackerman MJ. Plakophilin-2 truncation variants in patients clinically diagnosed with catecholaminergic polymorphic ventricular tachycardia and decedents with exercise-associated autopsy negative sudden unexplained death in the young. *JACC Clin Electrophysiol* 5: 120–127, 2019. doi:10.1016/j.jacep.2018.09.010.
364. Posch MG, Posch MJ, Geier C, Erdmann B, Mueller W, Richter A, Ruppert V, Pankuweit S, Maisch B, Perrot A, Buttgerit J, Dietz R, Haverkamp W, Ozelik C. A missense variant in desmoglein-2 predisposes to dilated cardiomyopathy. *Mol Genet Metab* 95: 74–80, 2008. doi:10.1016/j.ymgme.2008.06.005.
365. Brodehl A, Gaertner-Rommel A, Milting H. Molecular insights into cardiomyopathies associated with desmin (DES) mutations. *Biophys Rev* 10: 983–1006, 2018. doi:10.1007/s12551-018-0429-0.
366. Ferreira-Cornwell MC, Luo Y, Narula N, Lenox JM, Lieberman M, Radice GL. Remodeling the intercalated disc leads to cardiomyopathy in mice misexpressing cadherins in the heart. *J Cell Sci* 115: 1623–1634, 2002. doi:10.1242/jcs.115.8.1623.
367. Kostetskii I, Li J, Xiong Y, Zhou R, Ferrari VA, Patel VV, Molkentin JD, Radice GL. Induced deletion of the N-cadherin gene in the heart leads to dissolution of the intercalated disc structure. *Circ Res* 96: 346–354, 2005. doi:10.1161/01.RES.0000156274.72390.2c.

368. Li J, Patel VV, Kostetskii I, Xiong Y, Chu AF, Jacobson JT, Yu C, Morley GE, Molkentin JD, Radice GL. Cardiac-specific loss of N-cadherin leads to alteration in connexins with conduction slowing and arrhythmogenesis. *Circ Res* 97: 474–481, 2005. doi:10.1161/01.RES.0000181132.11393.18.
369. Sheikh F, Chen Y, Chen Y, Liang X, Hirschy A, Stenbit AE, Gu Y, Dalton ND, Yajima T, Lu Y, Knowlton KU, Peterson KL, Perriard JC, Chen J. alpha-E-catenin inactivation disrupts the cardiomyocyte adherens junction, resulting in cardiomyopathy and susceptibility to wall rupture. *Circulation* 114: 1046–1055, 2006. [Erratum in *Circulation* 114: e650, 2006]. doi:10.1161/CIRCULATIONAHA.106.634469.
370. Gustafson-Wagner EA, Sinn HW, Chen YL, Wang DZ, Reiter RS, Lin JL, Yang B, Williamson RA, Chen J, Lin CI, Lin JJ. Loss of mXlnalpha, an intercalated disk protein, results in cardiac hypertrophy and cardiomyopathy with conduction defects. *Am J Physiol Heart Circ Physiol* 293: H2680–H2692, 2007. doi:10.1152/ajpheart.00806.2007.
371. Van Norstrand DW, Asimaki A, Rubinos C, Dolmatova E, Srinivas M, Tester DJ, Saffitz JE, Duffy HS, Ackerman MJ. Connexin43 mutation causes heterogeneous gap junction loss and sudden infant death. *Circulation* 125: 474–481, 2012. doi:10.1161/CIRCULATIONAHA.111.057224.
372. Wu Q, Wu Y, Zhang L, Zheng J, Tang S, Cheng J. GJA1 gene variations in sudden unexplained nocturnal death syndrome in the Chinese Han population. *Forensic Sci Int* 270: 178–182, 2017. doi:10.1016/j.forsciint.2016.12.006.
373. Boente MD, Nanda A, Baselaga PA, Kelsell DP, McGrath JA, South AP. Cardiomyopathy diagnosed in the eldest child harbouring p. S24X mutation in JUP. *Br J Dermatol* 175: 644–646, 2016. doi:10.1111/bjd.14617.
374. Cabral RM, Liu L, Hogan C, Dopping-Hepenstal PJ, Winik BC, Asial RA, Dobson R, Mein CA, Baselaga PA, Mellerio JE, Nanda A, Boente Mdel C, Kelsell DP, McGrath JA, South AP. Homozygous mutations in the 5' region of the JUP gene result in cutaneous disease but normal heart development in children. *J Invest Dermatol* 130: 1543–1550, 2010. doi:10.1038/jid.2010.7.
375. Erken H, Yariz KO, Duman D, Kaya CT, Sayin T, Heper AO, Tekin M. Cardiomyopathy with alopecia and palmoplantar keratoderma (CAPK) is caused by a JUP mutation. *Br J Dermatol* 165: 917–921, 2011. doi:10.1111/j.1365-2133.2011.10455.x.
376. Lazzarini E, Jongbloed JD, Pillichou K, Thiene G, Basso C, Bikker H, Charbon B, Swertz M, van Tintelen JP, van der Zwaag PA. The ARVD/C genetic variants database: 2014 update. *Hum Mutat* 36: 403–410, 2015. doi:10.1002/humu.22765.
377. McKoy G, Protonotarios N, Crosby A, Tsatsopoulou A, Anastasakis A, Coonar A, Norman M, Baboonian C, Jeffery S, McKenna WJ. Identification of a deletion in plakoglobin in arrhythmogenic right ventricular cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease). *Lancet* 355: 2119–2124, 2000. doi:10.1016/S0140-6736(00)02379-5.
378. Oktem A, Doolan BJ, Akay BN, Onoufriadis A, Okcu Heper A, Kocak O, Ersoy-Evans S, McGrath JA. Autosomal recessive mutations in plakoglobin and risk of cardiac abnormalities. *Clin Exp Dermatol* 45: 654–657, 2020. doi:10.1111/ced.14201.
379. Pigors M, Kiritsi D, Krümpelmann S, Wagner N, He Y, Podda M, Kohlase J, Hausser J, Bruckner-Tuderman L, Has C. Lack of plakoglobin leads to lethal congenital epidermolysis bullosa: a novel clinico-genetic entity. *Hum Mol Genet* 20: 1811–1819, 2011. doi:10.1093/hmg/ddr064.
380. Protonotarios N, Tsatsopoulou A. Naxos disease: cardiocutaneous syndrome due to cell adhesion defect. *Orphanet J Rare Dis* 1: 4, 2006. doi:10.1186/1750-1172-1-4.
381. Rotemberg V, Garzon M, Lauren C, Iglesias A, Brachio SS, Aggarwal V, Stong N, Goldstein DB, Diacovo T. A novel mutation in junctional plakoglobin causing lethal congenital epidermolysis bullosa. *J Pediatr* 191: 266–269.e1, 2017. doi:10.1016/j.jpeds.2017.08.029.
382. Asimaki A, Syrris P, Wichter T, Matthias P, Saffitz JE, McKenna WJ. A novel dominant mutation in plakoglobin causes arrhythmogenic right ventricular cardiomyopathy. *Am J Hum Genet* 81: 964–973, 2007. doi:10.1086/521633.
383. Christensen AH, Benn M, Bundgaard H, Tybjaerg-Hansen A, Haunso S, Svendsen JH. Wide spectrum of desmosomal mutations in Danish patients with arrhythmogenic right ventricular cardiomyopathy. *J Med Genet* 47: 736–744, 2010. doi:10.1136/jmg.2010.077891.
384. den Haan AD, Tan BY, Zikusoka MN, Lladó LI, Jain R, Daly A, Tichnell C, James C, Amat-Alarcon N, Abraham T, Russell SD, Bluemke DA, Calkins H, Dalal D, Judge DP. Comprehensive desmosome mutation analysis in North Americans with arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circ Cardiovasc Genet* 2: 428–435, 2009. doi:10.1161/CIRCGENETICS.109.858217.
385. Liu L, Chen C, Li Y, Yu R. Whole-exome sequencing identified a de novo mutation of junction plakoglobin (p.R577C) in a Chinese patient with arrhythmogenic right ventricular cardiomyopathy. *Biomed Res Int* 2019: 9103860, 2019. doi:10.1155/2019/9103860.
386. Vahidnezhad H, Youssefian L, Faghankhani M, Mozafari N, Saeidian AH, Niaziroimi F, Abdollahimajd F, Sotoudeh S, Rajabi F, Mirsafaei L, Sani ZA, Liu L, Guy A, Zeinali S, Kariminejad A, Ho RT, McGrath JA, Uitto J. Arrhythmogenic right ventricular cardiomyopathy in patients with biallelic JUP-associated skin fragility. *Sci Rep* 10: 21622, 2020. doi:10.1038/s41598-020-78344-9.
387. Xu T, Yang Z, Vatta M, Rampazzo A, Boffagna G, Pillichou K, Pillichou K, Scherer SE, Saffitz J, Kravitz J, Zareba W, Danieli GA, Lorenzon A, Nava A, Baucé B, Thiene G, Basso C, Calkins H, Gear K, Marcus F, Towbin JA; Multidisciplinary Study of Right Ventricular Dysplasia Investigators. Compound and digenic heterozygosity contributes to arrhythmogenic right ventricular cardiomyopathy. *J Am Coll Cardiol* 55: 587–597, 2010. [Erratum in *J Am Coll Cardiol* 55: 1401, 2010].
388. Zhou X, Chen M, Song H, Wang B, Chen H, Wang J, Wang W, Feng S, Zhang F, Ju W, Li M, Gu K, Cao K, Wang DW, Yang B. Comprehensive analysis of desmosomal gene mutations in Han Chinese patients with arrhythmogenic right ventricular cardiomyopathy. *Eur J Med Genet* 58: 258–265, 2015. doi:10.1016/j.ejmg.2015.02.009.
389. Asimaki A, Tandri H, Huang H, Halushka MK, Gautam S, Basso C, Thiene G, Tsatsopoulou A, Protonotarios N, McKenna WJ, Calkins H, Saffitz JE. A new diagnostic test for arrhythmogenic right ventricular cardiomyopathy. *N Engl J Med* 360: 1075–1084, 2009. doi:10.1056/NEJMoa0808138.
390. Kant S, Krusche CA, Gaertner A, Milting H, Leube RE. Loss of plakoglobin immunoreactivity in intercalated discs in arrhythmogenic right ventricular cardiomyopathy: protein mislocalization versus epitope masking. *Cardiovasc Res* 109: 260–271, 2016. doi:10.1093/cvr/cvv270.
391. Swope D, Li J, Radice GL. Beyond cell adhesion: the role of armadillo proteins in the heart. *Cell Signal* 25: 93–100, 2013. doi:10.1016/j.cellsig.2012.09.025.
392. Knudsen KA, Wheelock MJ. Plakoglobin, or an 83-kD homologue distinct from beta-catenin, interacts with E-cadherin and N-cadherin. *J Cell Biol* 118: 671–679, 1992. doi:10.1083/jcb.118.3.671.
393. Sacco PA, McGranahan TM, Wheelock MJ, Johnson KR. Identification of plakoglobin domains required for association with N-cadherin and alpha-catenin. *J Biol Chem* 270: 20201–20206, 1995. doi:10.1074/jbc.270.34.20201.
394. Knudsen KA, Soler AP, Johnson KR, Wheelock MJ. Interaction of alpha-actinin with the cadherin/catenin cell-cell adhesion complex via alpha-catenin. *J Cell Biol* 130: 67–77, 1995. doi:10.1083/jcb.130.1.67.
395. Nieset JE, Redfield AR, Jin F, Knudsen KA, Johnson KR, Wheelock MJ. Characterization of the interactions of alpha-catenin with alpha-actinin and beta-catenin/plakoglobin. *J Cell Sci* 110: 1013–1022, 1997. doi:10.1242/jcs.110.8.1013.
396. Troyanovsky SM, Troyanovsky RB, Eshkind LG, Krutovskikh VA, Leube RE, Franke WW. Identification of the plakoglobin-binding domain in desmoglein and its role in plaque assembly and intermediate filament anchorage. *J Cell Biol* 127: 151–160, 1994. doi:10.1083/jcb.127.1.151.
397. Troyanovsky SM, Troyanovsky RB, Eshkind LG, Leube RE, Franke WW. Identification of amino acid sequence motifs in desmocollin, a desmosomal glycoprotein, that are required for plakoglobin binding and plaque formation. *Proc Natl Acad Sci USA* 91: 10790–10794, 1994. doi:10.1073/pnas.91.23.10790.
398. Kowalczyk AP, Bornslaeger EA, Borgwardt JE, Palka HL, Dhaliwal AS, Corcoran CM, Denning MF, Green KJ. The amino-terminal domain of desmoplakin binds to plakoglobin and clusters desmosomal

- cadherin-plakoglobin complexes. *J Cell Biol* 139: 773–784, 1997. doi:10.1083/jcb.139.3.773.
399. Ruiz P, Brinkmann V, Ledermann B, Behrend M, Grund C, Thalhammer C, Vogel F, Birchmeier C, Günthert U, Franke WW, Birchmeier W. Targeted mutation of plakoglobin in mice reveals essential functions of desmosomes in the embryonic heart. *J Cell Biol* 135: 215–225, 1996. doi:10.1083/jcb.135.1.215.
400. Bierkamp C, McLaughlin KJ, Schwarz H, Huber O, Kemler R. Embryonic heart and skin defects in mice lacking plakoglobin. *Dev Biol* 180: 780–785, 1996. doi:10.1006/dbio.1996.0346.
401. Isac CM, Ruiz P, Pfitzmaier B, Haase H, Birchmeier W, Morano I. Plakoglobin is essential for myocardial compliance but dispensable for myofibril insertion into adherens junctions. *J Cell Biochem* 72: 8–15, 1999. doi:10.1002/(SICI)1097-4644(199910)72:1<8::AID-JCB2>3.0.CO;2-A.
402. Kirchoff P, Fabritz L, Zwiener M, Witt H, Schäfers M, Zellerhoff S, Paul M, Athai T, Hiller KH, Baba HA, Breithardt G, Ruiz P, Wichter T, Levkau B. Age- and training-dependent development of arrhythmogenic right ventricular cardiomyopathy in heterozygous plakoglobin-deficient mice. *Circulation* 114: 1799–1806, 2006. doi:10.1161/CIRCULATIONAHA.106.624502.
403. Swope D, Li J, Muller EJ, Radice GL. Analysis of a Jup hypomorphic allele reveals a critical threshold for postnatal viability. *Genesis* 50: 717–727, 2012. doi:10.1002/dvg.22034.
404. Li D, Liu Y, Maruyama M, Zhu W, Chen H, Zhang W, Reuter S, Lin SF, Haneline LS, Field LJ, Chen PS, Shou W. Restrictive loss of plakoglobin in cardiomyocytes leads to arrhythmogenic cardiomyopathy. *Hum Mol Genet* 20: 4582–4596, 2011. doi:10.1093/hmg/ddr392.
405. Schinner C, Vielmuth F, Rötzer V, Hiermaier M, Radeva MY, Co TK, Hartlieb E, Schmidt A, Imhof A, Messoudi A, Horn A, Schlipp A, Spindler V, Waschke J. Adrenergic signaling strengthens cardiac myocyte cohesion. *Circ Res* 120: 1305–1317, 2017. doi:10.1161/CIRCRESAHA.116.309631.
406. Yeruva S, Kempf E, Egu DT, Flawinkel H, Kugelmann D, Waschke J. Adrenergic signaling-induced ultrastructural strengthening of intercalated discs via plakoglobin is crucial for positive adhesion in murine cardiomyocytes. *Front Physiol* 11: 430, 2020. doi:10.3389/fphys.2020.00430.
407. Schinner C, Erber BM, Yeruva S, Schlipp A, Rötzer V, Kempf E, Kant S, Leube RE, Mueller TD, Waschke J. Stabilization of desmoglein-2 binding rescues arrhythmia in arrhythmogenic cardiomyopathy. *JCI Insight* 5: e130141, 2020. doi:10.1172/jci.insight.130141.
408. Li J, Swope D, Raess N, Cheng L, Muller EJ, Radice GL. Cardiac tissue-restricted deletion of plakoglobin results in progressive cardiomyopathy and activation of beta-catenin signaling. *Mol Cell Biol* 31: 1134–1144, 2011. doi:10.1128/MCB.01025-10.
409. Swope D, Cheng L, Gao E, Li J, Radice GL. Loss of cadherin-binding proteins β -catenin and plakoglobin in the heart leads to gap junction remodeling and arrhythmogenesis. *Mol Cell Biol* 32: 1056–1067, 2012. doi:10.1128/MCB.06188-11.
410. Zhou J, Qu J, Yi XP, Graber K, Huber L, Wang X, Gerdes AM, Li F. Upregulation of gamma-catenin compensates for the loss of beta-catenin in adult cardiomyocytes. *Am J Physiol Heart Circ Physiol* 292: H270–H276, 2007. doi:10.1152/ajpheart.00576.2006.
411. Lombardi R, Dong J, Rodriguez G, Bell A, Leung TK, Schwartz RJ, Willerson JT, Brugada R, Marian AJ. Genetic fate mapping identifies second heart field progenitor cells as a source of adipocytes in arrhythmogenic right ventricular cardiomyopathy. *Circ Res* 104: 1076–1084, 2009. doi:10.1161/CIRCRESAHA.109.196899.
412. Lombardi R, da Graca Cabreira-Hansen M, Bell A, Fromm RR, Willerson JT, Marian AJ. Nuclear plakoglobin is essential for differentiation of cardiac progenitor cells to adipocytes in arrhythmogenic right ventricular cardiomyopathy. *Circ Res* 109: 1342–1353, 2011. doi:10.1161/CIRCRESAHA.111.255075.
413. Zhang Z, Stroud MJ, Zhang J, Fang X, Ouyang K, Kimura K, Mu Y, Dalton ND, Gu Y, Bradford WH, Peterson KL, Cheng H, Zhou X, Chen J. Normalization of Naxos plakoglobin levels restores cardiac function in mice. *J Clin Invest* 125: 1708–1712, 2015. doi:10.1172/JCI80335.
414. Bezzina CR, Pazoki R, Bardai A, Marsman RF, de Jong JS, Blom MT, Scicluna BP, Jukema JW, Bindraban NR, Lichtner P, Pfeufer A, Bishopric NH, Roden DM, Meitinger T, Chugh SS, Myerburg RJ, Jouven X, Käåb S, Dekker LR, Tan HL, Tanck MW, Wilde AA. Genome-wide association study identifies a susceptibility locus at 21q21 for ventricular fibrillation in acute myocardial infarction. *Nat Genet* 42: 688–691, 2010. doi:10.1038/ng.623.
415. Marsman RF, Bezzina CR, Freiberg F, Verkerk AO, Adriaens ME, Podliesna S, Chen C, Purfürst B, Spallek B, Koopmann TT, Baczko I, Dos Remedios CG, George AL Jr, Bishopric NH, Lodder EM, de Bakker JM, Fischer R, Coronel R, Wilde AA, Gotthardt M, Remme CA. Cxsackie and adenovirus receptor is a modifier of cardiac conduction and arrhythmia vulnerability in the setting of myocardial ischemia. *J Am Coll Cardiol* 63: 549–559, 2014. doi:10.1016/j.jacc.2013.10.062.
416. Shetty PB, Tang H, Tayo BO, Morrison AC, Hanis CL, Rao DC, Young JH, Fox ER, Boerwinkle E, Cooper RS, Risch NJ, Zhu X. Variants in CXADR and F2RL1 are associated with blood pressure and obesity in African-Americans in regions identified through admixture mapping. *J Hypertens* 30: 1970–1976, 2012.
417. Tomko RP, Xu R, Philipson L. HCAR and MCAR: the human and mouse cellular receptors for subgroup C adenoviruses and group B coxsackieviruses. *Proc Natl Acad Sci USA* 94: 3352–3356, 1997. doi:10.1073/pnas.94.7.3352.
418. Bergelson JM, Cunningham JA, Droguett G, Kurt-Jones EA, Krithivas A, Hong JS, Horwitz MS, Crowell RL, Finberg RW. Isolation of a common receptor for Cxsackie B viruses and adenoviruses 2 and 5. *Science* 275: 1320–1323, 1997. doi:10.1126/science.275.5304.1320.
419. Noutsias M, Fechner H, de Jonge H, Wang X, Dekkers D, Houtsmuller AB, Pauschinger M, Bergelson J, Warraich R, Yacoub M, Hetzer R, Lamers J, Schultheiss HP, Poller W. Human coxsackie-adenovirus receptor is colocalized with integrins $\alpha_5\beta_3$ and $\alpha_5\beta_1$ on the cardiomyocyte sarcolemma and upregulated in dilated cardiomyopathy: implications for cardiotropic viral infections. *Circulation* 104: 275–280, 2001. doi:10.1161/01.CIR.104.3.275.
420. Sasse A, Wallich M, Ding Z, Goedecke A, Schrader J. Cxsackie-adenovirus receptor mRNA expression in human heart failure. *J Gene Med* 5: 876–882, 2003. doi:10.1002/jgm.411.
421. Fechner H, Noutsias M, Tschoepe C, Hinze K, Wang X, Escher F, Pauschinger M, Dekkers D, Vetter R, Paul M, Lamers J, Schultheiss HP, Poller W. Induction of coxsackie-adenovirus-receptor expression during myocardial tissue formation and remodeling: identification of a cell-to-cell contact-dependent regulatory mechanism. *Circulation* 107: 876–882, 2003. doi:10.1161/01.CIR.0000050150.27478.C5.
422. Ito M, Kodama M, Masuko M, Yamaura M, Fuse K, Uesugi Y, Hirono S, Okura Y, Kato K, Hotta Y, Honda T, Kuwano R, Aizawa Y. Expression of coxsackie-adenovirus receptor in hearts of rats with experimental autoimmune myocarditis. *Circ Res* 86: 275–280, 2000. doi:10.1161/01.RES.86.3.275.
423. Cohen CJ, Shieh JT, Pickles RJ, Okegawa T, Hsieh JT, Bergelson JM. The coxsackie-adenovirus receptor is a transmembrane component of the tight junction. *Proc Natl Acad Sci USA* 98: 15191–15196, 2001. doi:10.1073/pnas.261452898.
424. Excoffon KJ, Hruska-Hageman A, Klotz M, Traver GL, Zabner J. A role for the PDZ-binding domain of the coxsackie B virus and adenovirus receptor (CAR) in cell adhesion and growth. *J Cell Sci* 117: 4401–4409, 2004. doi:10.1242/jcs.01300.
425. Chen JW, Zhou B, Yu QC, Shin SJ, Jiao K, Schneider MD, Baldwin HS, Bergelson JM. Cardiomyocyte-specific deletion of the coxsackie-adenovirus receptor results in hyperplasia of the embryonic left ventricle and abnormalities of sinuatrial valves. *Circ Res* 98: 923–930, 2006. doi:10.1161/01.RES.0000218041.41932.e3.
426. Dorner AA, Wegmann F, Butz S, Wolburg-Buchholz K, Wolburg H, Mack A, Nasdala I, August B, Westermann J, Rathjen FG, Vestweber D. Cxsackie-adenovirus receptor (CAR) is essential for early embryonic cardiac development. *J Cell Sci* 118: 3509–3521, 2005. doi:10.1242/jcs.02476.
427. Asher DR, Cerny AM, Weiler SR, Horner JW, Keeler ML, Neptune MA, Jones SN, Bronson RT, Depinho RA, Finberg RW. Cxsackie-adenovirus receptor is essential for cardiomyocyte development. *Genesis* 42: 77–85, 2005. doi:10.1002/gene.20127.
428. Jiao K, Kulessa H, Tompkins K, Zhou Y, Batts L, Baldwin HS, Hogan BL. An essential role of Bmp4 in the atrioventricular septation of the mouse heart. *Genes Dev* 17: 2362–2367, 2003. doi:10.1101/gad.1124803.
429. Lim BK, Xiong D, Dorner A, Youn TJ, Yung A, Liu TI, Gu Y, Dalton ND, Wright AT, Evans SM, Chen J, Peterson KL, McCulloch AD,

- Yajima T, Knowlton KU.** Coxsackievirus and adenovirus receptor (CAR) mediates atrioventricular-node function and connexin 45 localization in the murine heart. *J Clin Invest* 118: 2758–2770, 2008. doi:10.1172/JCI34777.
430. **Lisewski U, Shi Y, Wrackmeyer U, Fischer R, Chen C, Schirdewan A, Jüttner R, Rathjen F, Poller W, Radke MH, Gotthardt M.** The tight junction protein CAR regulates cardiac conduction and cell-cell communication. *J Exp Med* 205: 2369–2379, 2008. doi:10.1084/jem.20080897.
431. **Pazirandeh A, Sultana T, Mirza M, Rozell B, Hultenby K, Wallis K, Vennström B, Davis B, Arner A, Heuchel R, Löhr M, Philipson L, Sollerbrant K.** Multiple phenotypes in adult mice following inactivation of the Coxsackievirus and Adenovirus Receptor (Car) gene. *PLoS One* 6: e20203, 2011. doi:10.1371/journal.pone.0020203.
432. **Caruso L, Yuen S, Smith J, Husain M, Opavsky MA.** Cardiomyocyte-targeted overexpression of the coxsackie-adenovirus receptor causes a cardiomyopathy in association with beta-catenin signaling. *J Mol Cell Cardiol* 48: 1194–1205, 2010. doi:10.1016/j.yjmcc.2010.01.022.
433. **De Bortoli M, Postma AV, Poloni G, Calore M, Minervini G, Mazzotti E, Rigato I, Ebert M, Lorenzon A, Vazza G, Cipriani A, Bariani R, Marra MP, Husser D, Thiene G, Daliento L, Corrado D, Basso C, Tosatto SC, Baucé B, van Tintelen JP, Rampazzo A.** Whole-exome sequencing identifies pathogenic variants in TJP1 gene associated with arrhythmogenic cardiomyopathy. *Circ Genom Precis Med* 11: e002123, 2018. doi:10.1161/CIRCGEN.118.002123.
434. **Bauer H, Zweimueller-Mayer J, Steinbacher P, Lametschwandner A, Bauer HC.** The dual role of zonula occludens (ZO) proteins. *J Biomed Biotechnol* 2010: 402593, 2010. doi:10.1155/2010/402593.
435. **Toyofuku T, Yabuki M, Otsu K, Kuzuya T, Hori M, Tada M.** Direct association of the gap junction protein connexin-43 with ZO-1 in cardiac myocytes. *J Biol Chem* 273: 12725–12731, 1998. doi:10.1074/jbc.273.21.12725.
436. **Bruce AF, Rothery S, Dupont E, Severs NJ.** Gap junction remodeling in human heart failure is associated with increased interaction of connexin43 with ZO-1. *Cardiovasc Res* 77: 757–765, 2008. doi:10.1093/cvr/cvm083.
437. **Hunter AW, Barker RJ, Zhu C, Gourdie RG.** Zonula occludens-1 alters connexin43 gap junction size and organization by influencing channel accretion. *Mol Biol Cell* 16: 5686–5698, 2005. doi:10.1091/mbc.e05-08-0737.
438. **Itoh M, Nagafuchi A, Moroi S, Tsukita S.** Involvement of ZO-1 in cadherin-based cell adhesion through its direct binding to alpha catenin and actin filaments. *J Cell Biol* 138: 181–192, 1997. doi:10.1083/jcb.138.1.181.
439. **Fanning AS, Jameson BJ, Jesaitis LA, Anderson JM.** The tight junction protein ZO-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton. *J Biol Chem* 273: 29745–29753, 1998. doi:10.1074/jbc.273.45.29745.
440. **Fanning AS, Ma TY, Anderson JM.** Isolation and functional characterization of the actin binding region in the tight junction protein ZO-1. *FASEB J* 16: 1835–1837, 2002. doi:10.1096/fj.02-0121fje.
441. **Giepmans BN, Moolenaar WH.** The gap junction protein connexin43 interacts with the second PDZ domain of the zona occludens-1 protein. *Curr Biol* 8: 931–934, 1998. doi:10.1016/S0960-9822(07)00375-2.
442. **Giepmans BN, Verlaan I, Moolenaar WH.** Connexin-43 interactions with ZO-1 and alpha- and beta-tubulin. *Cell Commun Adhes* 8: 219–223, 2001. doi:10.3109/15419060109080727.
443. **Laing JG, Manley-Markowski RN, Koval M, Civitelli R, Steinberg TH.** Connexin45 interacts with zonula occludens-1 and connexin43 in osteoblastic cells. *J Biol Chem* 276: 23051–23055, 2001. doi:10.1074/jbc.M100303200.
444. **Kausalya PJ, Reichert M, Hunziker W.** Connexin45 directly binds to ZO-1 and localizes to the tight junction region in epithelial MDCK cells. *FEBS Lett* 505: 92–96, 2001. doi:10.1016/S0014-5793(01)02786-7.
445. **Zemljic-Harpf AE, Godoy JC, Platoshyn O, Asfaw EK, Busija AR, Domenighetti AA, Ross RS.** Vinculin directly binds zonula occludens-1 and is essential for stabilizing connexin-43-containing gap junctions in cardiac myocytes. *J Cell Sci* 127: 1104–1116, 2014. doi:10.1242/jcs.143743.
446. **Hunter AW, Jourdan J, Gourdie RG.** Fusion of GFP to the carboxyl terminus of connexin43 increases gap junction size in HeLa cells. *Cell Commun Adhes* 10: 211–214, 2003. doi:10.1080/cac.10.4-6.211.214.
447. **Rhett JM, Jourdan J, Gourdie RG.** Connexin 43 connexon to gap junction transition is regulated by zonula occludens-1. *Mol Biol Cell* 22: 1516–1528, 2011. doi:10.1091/mbc.e10-06-0548.
448. **Palatinus JA, O'Quinn MP, Barker RJ, Harris BS, Jourdan J, Gourdie RG.** ZO-1 determines adherens and gap junction localization at intercalated disks. *Am J Physiol Heart Circ Physiol* 300: H583–H594, 2011. doi:10.1152/ajpheart.00999.2010.
449. **Laing JG, Saffitz JE, Steinberg TH, Yamada KA.** Diminished zonula occludens-1 expression in the failing human heart. *Cardiovasc Pathol* 16: 159–164, 2007. doi:10.1016/j.carpath.2007.01.004.
450. **Kostin S.** Zonula occludens-1 and connexin 43 expression in the failing human heart. *J Cell Mol Med* 11: 892–895, 2007. doi:10.1111/j.1582-4934.2007.00063.x.
451. **Zhang J, Vincent KP, Peter AK, Klos M, Cheng H, Huang SM, Towne JK, Ferng D, Gu Y, Dalton ND, Chan Y, Li R, Peterson KL, Chen J, McCulloch AD, Knowlton KU, Ross RS.** Cardiomyocyte expression of ZO-1 is essential for normal atrioventricular conduction but does not alter ventricular function. *Circ Res* 127: 284–297, 2020. doi:10.1161/CIRCRESAHA.119.315539.
452. **Dai W, Nadadur RD, Brennan JA, Smith HL, Shen KM, Gadek M, Laforest B, Wang M, Gemel J, Li Y, Zhang J, Ziman BD, Yan J, Ai X, Beyer EC, Lakata EG, Kasthuri N, Efimov IR, Broman MT, Moskowitz IP, Shen L, Weber CR.** ZO-1 regulates intercalated disc composition and atrioventricular node conduction. *Circ Res* 127: e28–e43, 2020. doi:10.1161/CIRCRESAHA.119.316415.
453. **Henderson CA, Gomez CG, Novak SM, Mi-Mi L, Gregorio CC.** Overview of the muscle cytoskeleton. *Compr Physiol* 7: 891–944, 2017. doi:10.1002/cphy.c160033.
454. **Pardo JV, Siliciano JD, Craig SW.** A vinculin-containing cortical lattice in skeletal muscle: transverse lattice elements (“costameres”) mark sites of attachment between myofibrils and sarcolemma. *Proc Natl Acad Sci USA* 80: 1008–1012, 1983. doi:10.1073/pnas.80.4.1008.
455. **Gawor M, Prószyński TJ.** The molecular cross talk of the dystrophin-glycoprotein complex. *Ann NY Acad Sci* 1412: 62–72, 2018. doi:10.1111/nyas.13500.
456. **Gao QQ, McNally EM.** The dystrophin complex: structure, function, and implications for therapy. *Compr Physiol* 5: 1223–1239, 2015. doi:10.1002/cphy.c140048.
457. **Lapidos KA, Kakkar R, McNally EM.** The dystrophin glycoprotein complex: signaling strength and integrity for the sarcolemma. *Circ Res* 94: 1023–1031, 2004. doi:10.1161/01.RES.0000126574.61061.25.
458. **Jung D, Yang B, Meyer J, Chamberlain JS, Campbell KP.** Identification and characterization of the dystrophin anchoring site on beta-dystroglycan. *J Biol Chem* 270: 27305–27310, 1995. doi:10.1074/jbc.270.45.27305.
459. **Rybakova IN, Amann KJ, Ervasti JM.** A new model for the interaction of dystrophin with F-actin. *J Cell Biol* 135: 661–672, 1996. doi:10.1083/jcb.135.3.661.
460. **Rybakova IN, Patel JR, Ervasti JM.** The dystrophin complex forms a mechanically strong link between the sarcolemma and costameric actin. *J Cell Biol* 150: 1209–1214, 2000. doi:10.1083/jcb.150.5.1209.
461. **Ayalon G, Davis JQ, Scotland PB, Bennett V.** An ankyrin-based mechanism for functional organization of dystrophin and dystroglycan. *Cell* 135: 1189–1200, 2008. doi:10.1016/j.cell.2008.10.018.
462. **Manso AM, Li R, Monkley SJ, Cruz NM, Ong S, Lao DH, Koshman YE, Gu Y, Peterson KL, Chen J, Abel ED, Samarel AM, Critchley DR, Ross RS.** Talin1 has unique expression versus talin 2 in the heart and modifies the hypertrophic response to pressure overload. *J Biol Chem* 288: 4252–4264, 2013. doi:10.1074/jbc.M112.427484.
463. **Shai SY, Harpf AE, Babbitt CJ, Jordan MC, Fishbein MC, Chen J, Omura M, Leil TA, Becker KD, Jiang M, Smith DJ, Cherry SR, Loftus JC, Ross RS.** Cardiac myocyte-specific excision of the beta1 integrin gene results in myocardial fibrosis and cardiac failure. *Circ Res* 90: 458–464, 2002. doi:10.1161/hh0402.105790.
464. **Zhang Z, Mu Y, Veevers J, Peter AK, Manso AM, Bradford WH, Dalton ND, Peterson KL, Knowlton KU, Ross RS, Zhou X, Chen J.** Postnatal loss of kindlin-2 leads to progressive heart failure. *Circ Heart Fail* 9: e003129, 2016. doi:10.1161/CIRCHEARTFAILURE.116.003129.
465. **Zhang Z, Mu Y, Zhang J, Zhou Y, Cattaneo P, Veevers J, Peter AK, Manso AM, Knowlton KU, Zhou X, Evans SM, Ross RS, Chen J.** Kindlin-2 is essential for preserving integrity of the developing heart

- and preventing ventricular rupture. *Circulation* 139: 1554–1556, 2019. doi:10.1161/CIRCULATIONAHA.118.038383.
466. Dai X, Jiang W, Zhang Q, Xu L, Geng P, Zhuang S, Petrich BG, Jiang C, Peng L, Bhattacharya S, Evans SM, Sun Y, Chen J, Liang X. Requirement for integrin-linked kinase in neural crest migration and differentiation and outflow tract morphogenesis. *BMC Biol* 11: 107, 2013. doi:10.1186/1741-7007-11-107.
 467. Liang X, Sun Y, Chen J. Particularly interesting cysteine- and histidine-rich protein in cardiac development and remodeling. *J Investig Med* 57: 842–848, 2009. doi:10.2310/JIM.0b013e3181c5e31d.
 468. Liang X, Sun Y, Schneider J, Ding JH, Cheng H, Ye M, Bhattacharya S, Rearden A, Evans S, Chen J. Pinch1 is required for normal development of cranial and cardiac neural crest-derived structures. *Circ Res* 100: 527–535, 2007. doi:10.1161/01.RES.0000259041.37059.8c.
 469. Liang X, Sun Y, Ye M, Scimia MC, Cheng H, Martin J, Wang G, Rearden A, Wu C, Peterson KL, Powell HC, Evans SM, Chen J. Targeted ablation of PINCH1 and PINCH2 from murine myocardium results in dilated cardiomyopathy and early postnatal lethality. *Circulation* 120: 568–576, 2009. doi:10.1161/CIRCULATIONAHA.109.864686.
 470. Liang X, Zhou Q, Li X, Sun Y, Lu M, Dalton N, Ross J Jr, Chen J. PINCH1 plays an essential role in early murine embryonic development but is dispensable in ventricular cardiomyocytes. *Mol Cell Biol* 25: 3056–3062, 2005. doi:10.1128/MCB.25.8.3056-3062.2005.
 471. Mao Z, Nakamura F. Structure and function of filamin C in the muscle Z-disc. *Int J Mol Sci* 21: 2696, 2020. doi:10.3390/ijms21082696.
 472. D'Amario D, Gowran A, Canonico F, Castiglioni E, Rovina D, Santoro R, Spinelli P, Adoriso R, Amodeo A, Perrucci GL, Borovac JA, Pompilio G, Crea F. Dystrophin cardiomyopathies: clinical management, molecular pathogenesis and evolution towards precision medicine. *J Clin Med* 7: 291, 2018. doi:10.3390/jcm7090291.
 473. Florczyk-Soluch U, Polak K, Dulak J. The multifaceted view of heart problem in Duchenne muscular dystrophy. *Cell Mol Life Sci* 78: 5447–5468, 2021. doi:10.1007/s00018-021-03862-2.
 474. Schade van Westrum SM, Dekker LR, de Voogt WG, Wilde AA, Ginjaar IB, de Visser M, van der Kooij AJ. Cardiac involvement in Dutch patients with sarcoglycanopathy: a cross-sectional cohort and follow-up study. *Muscle Nerve* 50: 909–913, 2014. doi:10.1002/mus.24233.
 475. Faysoil A. Cardiac diseases in sarcoglycanopathies. *Int J Cardiol* 144: 67–68, 2010. doi:10.1016/j.ijcard.2008.12.048.
 476. Kärkkäinen S, Miettinen R, Tuomainen P, Kärkkäinen P, Helio T, Reissell E, Kaartinen M, Toivonen L, Nieminen MS, Kuusisto J, Laakso M, Peuhkurinen K. A novel mutation, Arg71Thr, in the delta-sarcoglycan gene is associated with dilated cardiomyopathy. *J Mol Med (Berl)* 81: 795–800, 2003. doi:10.1007/s00109-003-0480-5.
 477. Tsubata S, Bowles KR, Vatta M, Zintz C, Titus J, Muhonen L, Bowles NE, Towbin JA. Mutations in the human delta-sarcoglycan gene in familial and sporadic dilated cardiomyopathy. *J Clin Invest* 106: 655–662, 2000. doi:10.1172/JCI9224.
 478. Cao Q, Shen Y, Liu X, Yu X, Yuan P, Wan R, Liu X, Peng X, He W, Pu J, Hong K. Phenotype and functional analyses in a transgenic mouse model of left ventricular noncompaction caused by a DTNA mutation. *Int Heart J* 58: 939–947, 2017. doi:10.1536/ihj.16-019.
 479. Ichida F, Tsubata S, Bowles KR, Haneda N, Uese K, Miyawaki T, Dreyer WJ, Messina J, Li H, Bowles NE, Towbin JA. Novel gene mutations in patients with left ventricular noncompaction or Barth syndrome. *Circulation* 103: 1256–1263, 2001. doi:10.1161/01.CIR.103.9.1256.
 480. Ueda K, Valdivia C, Medeiros-Domingo A, Tester DJ, Vatta M, Farrugia G, Ackerman MJ, Makielski JC. Syntrophin mutation associated with long QT syndrome through activation of the nNOS-SCN5A macromolecular complex. *Proc Natl Acad Sci USA* 105: 9355–9360, 2008. doi:10.1073/pnas.0801294105.
 481. Hawley MH, Almontashiri N, Biesecker LG, Berger N, Chung WK, Garcia J, Grebe TA, Kelly MA, Lebo MS, Macaya D, Mei H, Platt J, Richard G, Ryan A, Thomson KL, Vatta M, Walsh R, Ware JS, Wheeler M, Zouk H, Mason-Suares H, Funke B. An assessment of the role of vinculin loss of function variants in inherited cardiomyopathy. *Hum Mutat* 41: 1577–1587, 2020. doi:10.1002/humu.24061.
 482. Hall CL, Gurha P, Sabater-Molina M, Asimaki A, Futema M, Lovering RC, Suárez MP, Aguilera B, Molina P, Zorio E, Coarfa C, Robertson MJ, Cheedipudi SM, Ng KE, Delaney P, Hernández JP, Pastor F, Gimeno JR, McKenna WJ, Marian AJ, Syrris P. RNA sequencing-based transcriptome profiling of cardiac tissue implicates novel putative disease mechanisms in FLNC-associated arrhythmogenic cardiomyopathy. *Int J Cardiol* 302: 124–130, 2020. doi:10.1016/j.ijcard.2019.12.002.
 483. Knöll R, Postel R, Wang J, Krätzner R, Hennecke G, Vacaru AM, Vakeel P, Schubert C, Murthy K, Rana BK, Kube D, Knöll G, Schäfer K, Hayashi T, Holm T, Kimura A, Schork N, Toliat MR, Nürnberg P, Schultheiss HP, Schaper W, Schaper J, Bos E, Den Hertog J, van Eeden FJ, Peters PJ, Hasenfuss G, Chien KR, Bakkers J. Laminin-alpha4 and integrin-linked kinase mutations cause human cardiomyopathy via simultaneous defects in cardiomyocytes and endothelial cells. *Circulation* 116: 515–525, 2007. doi:10.1161/CIRCULATIONAHA.107.689984.
 484. Song S, Shi A, Lian H, Hu S, Nie Y. Filamin C in cardiomyopathy: from physiological roles to DNA variants. *Heart Fail Rev*. In press. doi:10.1007/s10741-021-10172-z.
 485. Maeda M, Holder E, Lowes B, Valent S, Bies RD. Dilated cardiomyopathy associated with deficiency of the cytoskeletal protein metavinculin. *Circulation* 95: 17–20, 1997. doi:10.1161/01.CIR.95.1.17.
 486. Vasile VC, Will ML, Ommen SR, Edwards WD, Olson TM, Ackerman MJ. Identification of a metavinculin missense mutation, R975W, associated with both hypertrophic and dilated cardiomyopathy. *Mol Genet Metab* 87: 169–174, 2006. doi:10.1016/j.ymgme.2005.08.006.
 487. Olson TM, Illenberger S, Kishimoto NY, Huttelmaier S, Keating MT, Jockusch BM. Metavinculin mutations alter actin interaction in dilated cardiomyopathy. *Circulation* 105: 431–437, 2002. doi:10.1161/hc0402.102930.
 488. 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* 345: 998–1003, 2006. doi:10.1016/j.bbrc.2006.04.151.
 489. Vasile VC, Edwards WD, Ommen SR, Ackerman MJ. Obstructive hypertrophic cardiomyopathy is associated with reduced expression of vinculin in the intercalated disc. *Biochem Biophys Res Commun* 349: 709–715, 2006. doi:10.1016/j.bbrc.2006.08.106.
 490. Wells QS, Ausborn NL, Funke BH, Pfothenauer JP, Fredi JL, Baxter S, Disalvo TD, Hong CC. Familial dilated cardiomyopathy associated with congenital defects in the setting of a novel VCL mutation (Lys815Arg) in conjunction with a known MYPBC3 variant. *Cardiogenetics* 1: e10, 2011. doi:10.4081/cardiogenetics.2011.e10.
 491. Deacon DC, Happe CL, Chen C, Tedeschi N, Manso AM, Li T, Dalton ND, Peng Q, Farah EN, Gu Y, Tenerelli KP, Tran VD, Chen J, Peterson KL, Schork NJ, Adler ED, Engler AJ, Ross RS, Chi NC. Combinatorial interactions of genetic variants in human cardiomyopathy. *Nat Biomed Eng* 3: 147–157, 2019. doi:10.1038/s41551-019-0348-9.
 492. Geiger B, Tokuyasu KT, Dutton AH, Singer SJ. Vinculin, an intracellular protein localized at specialized sites where microfilament bundles terminate at cell membranes. *Proc Natl Acad Sci USA* 77: 4127–4131, 1980. doi:10.1073/pnas.77.7.4127.
 493. Belkin AM, Ornatsky OI, Glukhova MA, Koteliansky VE. Immunolocalization of meta-vinculin in human smooth and cardiac muscles. *J Cell Biol* 107: 545–553, 1988. doi:10.1083/jcb.107.2.545.
 494. Burridge K, Mangeat P. An interaction between vinculin and talin. *Nature* 308: 744–746, 1984. doi:10.1038/308744a0.
 495. Johnson RP, Craig SW. An intramolecular association between the head and tail domains of vinculin modulates talin binding. *J Biol Chem* 269: 12611–12619, 1994. doi:10.1016/S0021-9258(18)99920-5.
 496. Bass MD, Smith BJ, Prigent SA, Critchley DR. Talin contains three similar vinculin-binding sites predicted to form an amphipathic helix. *Biochem J* 341: 257–263, 1999. doi:10.1042/bj3410257.
 497. Kroemker M, Rüdiger AH, Jockusch BM, Rüdiger M. Intramolecular interactions in vinculin control alpha-actinin binding to the vinculin head. *FEBS Lett* 355: 259–262, 1994. doi:10.1016/0014-5793(94)01216-4.
 498. Bois PR, Borgon RA, Vonnrhein C, Izard T. Structural dynamics of alpha-actinin-vinculin interactions. *Mol Cell Biol* 25: 6112–6122, 2005. [Erratum in *Mol Cell Biol* 27: 5606, 2007]. doi:10.1128/MCB.25.14.6112-6122.2005.
 499. Weiss EE, Kroemker M, Rüdiger AH, Jockusch BM, Rüdiger M. Vinculin is part of the cadherin-catenin junctional complex: complex formation between alpha-catenin and vinculin. *J Cell Biol* 141: 755–764, 1998. doi:10.1083/jcb.141.3.755.

500. **Watabe-Uchida M, Uchida N, Imamura Y, Nagafuchi A, Fujimoto K, Uemura T, Vermeulen S, van Roy F, Adamson ED, Takeichi M.** alpha-Catenin-vinculin interaction functions to organize the apical junctional complex in epithelial cells. *J Cell Biol* 142: 847–857, 1998. doi:10.1083/jcb.142.3.847.
501. **Yamada S, Pokutta S, Drees F, Weis WI, Nelson WJ.** Deconstructing the cadherin-catenin-actin complex. *Cell* 123: 889–901, 2005. doi:10.1016/j.cell.2005.09.020.
502. **Hazan RB, Kang L, Roe S, Borgen PI, Rimm DL.** Vinculin is associated with the E-cadherin adhesion complex. *J Biol Chem* 272: 32448–32453, 1997. doi:10.1074/jbc.272.51.32448.
503. **Brindle NP, Holt MR, Davies JE, Price CJ, Critchley DR.** The focal-adhesion vasodilator-stimulated phosphoprotein (VASP) binds to the proline-rich domain in vinculin. *Biochem J* 318: 753–757, 1996. doi:10.1042/bj3180753.
504. **Kioka N, Sakata S, Kawauchi T, Amachi T, Akiyama SK, Okazaki K, Yaen C, Yamada KM, Aota S.** Vinexin: a novel vinculin-binding protein with multiple SH3 domains enhances actin cytoskeletal organization. *J Cell Biol* 144: 59–69, 1999. doi:10.1083/jcb.144.1.59.
505. **Mandai K, Nakanishi H, Satoh A, Takahashi K, Satoh K, Nishioka H, Mizoguchi A, Takai Y.** Ponsin/SH3P12: an I-fafadin- and vinculin-binding protein localized at cell-cell and cell-matrix adherens junctions. *J Cell Biol* 144: 1001–1017, 1999. doi:10.1083/jcb.144.5.1001.
506. **DeMali KA, Barlow CA, Burrridge K.** Recruitment of the Arp2/3 complex to vinculin: coupling membrane protrusion to matrix adhesion. *J Cell Biol* 159: 881–891, 2002. doi:10.1083/jcb.200206043.
507. **Johnson RP, Niggli V, Durrer P, Craig SW.** A conserved motif in the tail domain of vinculin mediates association with and insertion into acidic phospholipid bilayers. *Biochemistry* 37: 10211–10222, 1998. doi:10.1021/bi9727242.
508. **Hüttelmaier S, Mayboroda O, Harbeck B, Jarchau T, Jockusch BM, Rüdiger M.** The interaction of the cell-contact proteins VASP and vinculin is regulated by phosphatidylinositol-4,5-bisphosphate. *Curr Biol* 8: 479–488, 1998. doi:10.1016/S0960-9822(98)70199-X.
509. **Turner CE, Glenney JR Jr, Burrridge K.** Paxillin: a new vinculin-binding protein present in focal adhesions. *J Cell Biol* 111: 1059–1068, 1990. doi:10.1083/jcb.111.3.1059.
510. **Wood CK, Turner CE, Jackson P, Critchley DR.** Characterisation of the paxillin-binding site and the C-terminal focal adhesion targeting sequence in vinculin. *J Cell Sci* 107: 709–717, 1994. doi:10.1242/jcs.107.2.709.
511. **Hüttelmaier S, Illenberger S, Grosheva I, Rüdiger M, Singer RH, Jockusch BM.** Raver1, a dual compartment protein, is a ligand for PTB/hnRNPI and microfilament attachment proteins. *J Cell Biol* 155: 775–786, 2001. doi:10.1083/jcb.200105044.
512. **Sun N, Critchley DR, Paulin D, Li Z, Robson RM.** Human alpha-synemin interacts directly with vinculin and metavinculin. *Biochem J* 409: 657–667, 2008. doi:10.1042/BJ20071188.
513. **Ziegler WH, Tigges U, Zieseniss A, Jockusch BM.** A lipid-regulated docking site on vinculin for protein kinase C. *J Biol Chem* 277: 7396–7404, 2002. doi:10.1074/jbc.M110008200.
514. **Menkel AR, Kroemker M, Bubeck P, Ronsiek M, Nikolai G, Jockusch BM.** Characterization of an F-actin-binding domain in the cytoskeletal protein vinculin. *J Cell Biol* 126: 1231–1240, 1994. doi:10.1083/jcb.126.5.1231.
515. **Hüttelmaier S, Bubeck P, Rüdiger M, Jockusch BM.** Characterization of two F-actin-binding and oligomerization sites in the cell-contact protein vinculin. *Eur J Biochem* 247: 1136–1142, 1997. doi:10.1111/j.1432-1033.1997.01136.x.
516. **Ruhnau K, Wegner A.** Evidence for direct binding of vinculin to actin filaments. *FEBS Lett* 228: 105–108, 1988. doi:10.1016/0014-5793(88)80595-7.
517. **Jockusch BM, Isenberg G.** Interaction of alpha-actinin and vinculin with actin: opposite effects on filament network formation. *Proc Natl Acad Sci USA* 78: 3005–3009, 1981. doi:10.1073/pnas.78.5.3005.
518. **Bays JL, DeMali KA.** Vinculin in cell-cell and cell-matrix adhesions. *Cell Mol Life Sci* 74: 2999–3009, 2017. doi:10.1007/s00018-017-2511-3.
519. **Lu MH, DiLullo C, Schultheiss T, Holtzer S, Murray JM, Choi J, Fischman DA, Holtzer H.** The vinculin/sarcomeric-alpha-actinin/alpha-actin nexus in cultured cardiac myocytes. *J Cell Biol* 117: 1007–1022, 1992. doi:10.1083/jcb.117.5.1007.
520. **Merkel CD, Li Y, Raza Q, Stoilz DB, Kwiatkowski AV.** Vinculin anchors contractile actin to the cardiomyocyte adherens junction. *Mol Biol Cell* 30: 2639–2650, 2019. doi:10.1091/mbc.E19-04-0216.
521. **Anastasi G, Cutroneo G, Gaeta R, Di Mauro D, Arco A, Consolo A, Santoro G, Trimarchi F, Favalaro A.** Dystrophin-glycoprotein complex and vinculin-talin-integrin system in human adult cardiac muscle. *Int J Mol Med* 23: 149–159, 2009.
522. **Wu JC, Sung HC, Chung TH, DePhilip RM.** Role of N-cadherin- and integrin-based costameres in the development of rat cardiomyocytes. *J Cell Biochem* 84: 717–724, 2002. doi:10.1002/jcb.10092.
523. **Moiseyeva EP, Weller PA, Zhidkova NI, Corben EB, Patel B, Jasinska I, Koteliensky VE, Critchley DR.** Organization of the human gene encoding the cytoskeletal protein vinculin and the sequence of the vinculin promoter. *J Biol Chem* 268: 4318–4325, 1993. doi:10.1016/S0021-9258(18)53612-7.
524. **Feramisco JR, Smart JE, Burrridge K, Helfman DM, Thomas GP.** Coexistence of vinculin and a vinculin-like protein of higher molecular weight in smooth muscle. *J Biol Chem* 257: 11024–11031, 1982. doi:10.1016/S0021-9258(18)33927-9.
525. **Saga S, Hamaguchi M, Hoshino M, Kojima K.** Expression of meta-vinculin associated with differentiation of chicken embryonal muscle cells. *Exp Cell Res* 156: 45–56, 1985. doi:10.1016/0014-4827(85)90260-5.
526. **Belkin AM, Ornatsky OI, Kabakov AE, Glukhova MA, Koteliensky VE.** Diversity of vinculin/meta-vinculin in human tissues and cultivated cells. Expression of muscle specific variants of vinculin in human aorta smooth muscle cells. *J Biol Chem* 263: 6631–6635, 1988. doi:10.1016/S0021-9258(18)68688-0.
527. **Janssen ME, Liu H, Volkmann N, Hanein D.** The C-terminal tail domain of metavinculin, vinculin's splice variant, severs actin filaments. *J Cell Biol* 197: 585–593, 2012. doi:10.1083/jcb.201111046.
528. **Kim LY, Thompson PM, Lee HT, Pershad M, Campbell SL, Alushin GM.** The structural basis of actin organization by vinculin and meta-vinculin. *J Mol Biol* 428: 10–25, 2016. doi:10.1016/j.jmb.2015.09.031.
529. **Kanoldt V, Kluger C, Barz C, Schweizer AL, Ramanujam D, Windgasse L, Engelhardt S, Chrostek-Grashoff A, Grashoff C.** Metavinculin modulates force transduction in cell adhesion sites. *Nat Commun* 11: 6403, 2020. doi:10.1038/s41467-020-20125-z.
530. **Xu W, Baribault H, Adamson ED.** Vinculin knockout results in heart and brain defects during embryonic development. *Development* 125: 327–337, 1998. doi:10.1242/dev.125.2.327.
531. **Zemljic-Harpf AE, Ponrartana S, Avalos RT, Jordan MC, Roos KP, Dalton ND, Phan VQ, Adamson ED, Ross RS.** Heterozygous inactivation of the vinculin gene predisposes to stress-induced cardiomyopathy. *Am J Pathol* 165: 1033–1044, 2004. doi:10.1016/S0002-9440(10)63364-0.
532. **Zemljic-Harpf AE, Miller JC, Henderson SA, Wright AT, Manso AM, Elsherif L, Dalton ND, Thor AK, Perkins GA, McCulloch AD, Ross RS.** Cardiac-myocyte-specific excision of the vinculin gene disrupts cellular junctions, causing sudden death or dilated cardiomyopathy. *Mol Cell Biol* 27: 7522–7537, 2007. doi:10.1128/MCB.00728-07.
533. **Brodehl A, Rezazadeh S, Williams T, Munsie NM, Liedtke D, Oh T, Ferrier R, Shen Y, Jones SJ, Stiegler AL, Boggan TJ, Duff HJ, Friedman JM, Gibson WT, Consortium FC, Childs SJ, Gerull B; FORGE Canada Consortium.** Mutations in ILK, encoding integrin-linked kinase, are associated with arrhythmogenic cardiomyopathy. *Transl Res* 208: 15–29, 2019. doi:10.1016/j.trsl.2019.02.004.
534. **Meder B, Haas J, Keller A, Heid C, Just S, Borries A, Boisguerin V, Scharfenberger-Schmeer M, Stähler P, Beier M, Weichenhan D, Strom TM, Pfeufer A, Korn B, Katus HA, Rottbauer W.** Targeted next-generation sequencing for the molecular genetic diagnostics of cardiomyopathies. *Circ Cardiovasc Genet* 4: 110–122, 2011. doi:10.1161/CIRCGENETICS.110.958322.
535. **Haas J, Frese KS, Peil B, Kloos W, Keller A, Nietsch R, Feng Z, et al.** Atlas of the clinical genetics of human dilated cardiomyopathy. *Eur Heart J* 36: 1123–1135a, 2015. doi:10.1093/eurheartj/ehu301.
536. **Lu H, Fedak PW, Dai X, Du C, Zhou YQ, Henkelman M, Mongroo PS, Lau A, Yamabi H, Hinek A, Husain M, Hannigan G, Coles JG.** Integrin-linked kinase expression is elevated in human cardiac hypertrophy and induces hypertrophy in transgenic mice. *Circulation* 114: 2271–2279, 2006. doi:10.1161/CIRCULATIONAHA.106.642330.
537. **Legate KR, Montañez E, Kudlacek O, Fässler R.** PINCH and parvin: the tIPP of integrin signalling. *Nat Rev Mol Cell Biol* 7: 20–31, 2006. doi:10.1038/nrm1789.

538. **Ghatak S, Morgner J, Wickström SA.** ILK: a pseudokinase with a unique function in the integrin-actin linkage. *Biochem Soc Trans* 41: 995–1001, 2013. doi:10.1042/BST20130062.
539. **Dedhar S, Williams B, Hannigan G.** Integrin-linked kinase (ILK): a regulator of integrin and growth-factor signalling. *Trends Cell Biol* 9: 319–323, 1999. doi:10.1016/S0962-8924(99)01612-8.
540. **Tu Y, Li F, Goicoechea S, Wu C.** The LIM-only protein PINCH directly interacts with integrin-linked kinase and is recruited to integrin-rich sites in spreading cells. *Mol Cell Biol* 19: 2425–2434, 1999. doi:10.1128/MCB.19.3.2425.
541. **Li F, Zhang Y, Wu C.** Integrin-linked kinase is localized to cell-matrix focal adhesions but not cell-cell adhesion sites and the focal adhesion localization of integrin-linked kinase is regulated by the PINCH-binding ANK repeats. *J Cell Sci* 112: 4589–4599, 1999. doi:10.1242/jcs.112.24.4589.
542. **Bock-Marquette I, Saxena A, White MD, Dimaio JM, Srivastava D.** Thymosin beta4 activates integrin-linked kinase and promotes cardiac cell migration, survival and cardiac repair. *Nature* 432: 466–472, 2004. doi:10.1038/nature03000.
543. **Yamazaki T, Masuda J, Omori T, Usui R, Akiyama H, Maru Y.** EphA1 interacts with integrin-linked kinase and regulates cell morphology and motility. *J Cell Sci* 122: 243–255, 2009. doi:10.1242/jcs.036467.
544. **Leung-Hageteijn C, Mahendra A, Naruszewicz I, Hannigan GE.** Modulation of integrin signal transduction by ILKAP, a protein phosphatase 2C associating with the integrin-linked kinase, ILK1. *EMBO J* 20: 2160–2170, 2001. doi:10.1093/emboj/20.9.2160.
545. **Nakrieko KA, Vespa A, Mason D, Irvine TS, D'Souza SJ, Dagnino L.** Modulation of integrin-linked kinase nucleo-cytoplasmic shuttling by ILKAP and CRM1. *Cell Cycle* 7: 2157–2166, 2008. doi:10.4161/cc.7.14.6241.
546. **Hannigan GE, Leung-Hageteijn C, Fitz-Gibbon L, Coppolino MG, Radeva G, Filmus J, Bell JC, Dedhar S.** Regulation of cell adhesion and anchorage-dependent growth by a new beta 1-integrin-linked protein kinase. *Nature* 379: 91–96, 1996. doi:10.1038/379091a0.
547. **Pasquet JM, Noury M, Nurden AT.** Evidence that the platelet integrin alphaIIb beta3 is regulated by the integrin-linked kinase, ILK, in a PI3-kinase dependent pathway. *Thromb Haemost* 88: 115–122, 2002. doi:10.1055/s-0037-1613163.
548. **Fukuda K, Gupta S, Chen K, Wu C, Qin J.** The pseudoactive site of ILK is essential for its binding to alpha-Parvin and localization to focal adhesions. *Mol Cell* 36: 819–830, 2009. doi:10.1016/j.molcel.2009.11.028.
549. **Tu Y, Huang Y, Zhang Y, Hua Y, Wu C.** A new focal adhesion protein that interacts with integrin-linked kinase and regulates cell adhesion and spreading. *J Cell Biol* 153: 585–598, 2001. doi:10.1083/jcb.153.3.585.
550. **Yamaji S, Suzuki A, Sugiyama Y, Koide Y, Yoshida M, Kanamori H, Mohri H, Ohno S, Ishigatsubo Y.** A novel integrin-linked kinase-binding protein, affixin, is involved in the early stage of cell-substrate interaction. *J Cell Biol* 153: 1251–1264, 2001. doi:10.1083/jcb.153.6.1251.
551. **Nikolopoulos SN, Turner CE.** Molecular dissection of actopaxin-integrin-linked kinase-Paxillin interactions and their role in subcellular localization. *J Biol Chem* 277: 1568–1575, 2002. doi:10.1074/jbc.M108612200.
552. **He J, Li Y, Wei S, Guo M, Fu W.** [Effects of mixture of Astragalus membranaceus, Fructus Ligustri lucidi and Eclipta prostrata on immune function in mice]. *Hua Xi Yi Ke Da Xue Xue Bao* 23: 408–411, 1992.
553. **Nikolopoulos SN, Turner CE.** Integrin-linked kinase (ILK) binding to paxillin LD1 motif regulates ILK localization to focal adhesions. *J Biol Chem* 276: 23499–23505, 2001. doi:10.1074/jbc.M102163200.
554. **Huet-Calderwood C, Brahme NN, Kumar N, Stiegler AL, Raghavan S, Boggon TJ, Calderwood DA.** Differences in binding to the ILK complex determines kindlin isoform adhesion localization and integrin activation. *J Cell Sci* 127: 4308–4321, 2014. doi:10.1242/jcs.155879.
555. **Mackinnon AC, Qadota H, Norman KR, Moerman DG, Williams BD.** C. elegans PAT-4/ILK functions as an adaptor protein within integrin adhesion complexes. *Curr Biol* 12: 787–797, 2002. doi:10.1016/S0960-9822(02)00810-2.
556. **McDonald PC, Oloumi A, Mills J, Dobreva I, Maidan M, Gray V, Wederell ED, Bally MB, Foster LJ, Dedhar S.** Rictor and integrin-linked kinase interact and regulate Akt phosphorylation and cancer cell survival. *Cancer Res* 68: 1618–1624, 2008. doi:10.1158/0008-5472.CAN-07-5869.
557. **Persad S, Attwell S, Gray V, Mawji N, Deng JT, Leung D, Yan J, Sanghera J, Walsh MP, Dedhar S.** Regulation of protein kinase B/Akt-serine 473 phosphorylation by integrin-linked kinase: critical roles for kinase activity and amino acids arginine 211 and serine 343. *J Biol Chem* 276: 27462–27469, 2001. doi:10.1074/jbc.M102940200.
558. **Kim YB, Choi S, Choi MC, Oh MA, Lee SA, Cho M, Mizuno K, Kim SH, Lee JW.** Cell adhesion-dependent cofilin serine 3 phosphorylation by the integrin-linked kinase-c-Src complex. *J Biol Chem* 283: 10089–10096, 2008. doi:10.1074/jbc.M708300200.
559. **Delcommenne M, Tan C, Gray V, Rue L, Woodgett J, Dedhar S.** Phosphoinositide-3-OH kinase-dependent regulation of glycogen synthase kinase 3 and protein kinase B/AKT by the integrin-linked kinase. *Proc Natl Acad Sci USA* 95: 11211–11216, 1998. doi:10.1073/pnas.95.19.11211.
560. **Hannigan GE, McDonald PC, Walsh MP, Dedhar S.** Integrin-linked kinase: not so 'pseudo' after all. *Oncogene* 30: 4375–4385, 2011. doi:10.1038/onc.2011.177.
561. **Wickström SA, Lange A, Montanez E, Fässler R.** The ILK/PINCH/parvin complex: the kinase is dead, long live the pseudokinase! *EMBO J* 29: 281–291, 2010. doi:10.1038/emboj.2009.376.
562. **Fukuda K, Knight JD, Piszczek G, Kothary R, Qin J.** Biochemical, proteomic, structural, and thermodynamic characterizations of integrin-linked kinase (ILK): cross-validation of the pseudokinase. *J Biol Chem* 286: 21886–21895, 2011. doi:10.1074/jbc.M111.240093.
563. **Bulus N, Brown KL, Mernaugh G, Böttcher A, Dong X, Sanders CR, Pozzi A, Fässler R, Zent R.** Disruption of the integrin-linked kinase (ILK) pseudokinase domain affects kidney development in mice. *J Biol Chem* 296: 100361, 2021. doi:10.1016/j.jbc.2021.100361.
564. **Sakai T, Li S, Docheva D, Grashoff C, Sakai K, Kostka G, Braun A, Pfeifer A, Yurchenco PD, Fässler R.** Integrin-linked kinase (ILK) is required for polarizing the epiblast, cell adhesion, and controlling actin accumulation. *Genes Dev* 17: 926–940, 2003. doi:10.1101/gad.255603.
565. **White DE, Coutu P, Shi YF, Tardif JC, Nattel S, St Arnaud R, Dedhar S, Muller WJ.** Targeted ablation of ILK for the murine heart results in dilated cardiomyopathy and spontaneous heart failure. *Genes Dev* 20: 2355–2360, 2006. doi:10.1101/gad.1458906.
566. **Quang KL, Maguy A, Qi XY, Naud P, Xiong F, Tadevosyan A, Shi YF, Chartier D, Tardif JC, Dobrev D, Nattel S.** Loss of cardiomyocyte integrin-linked kinase produces an arrhythmogenic cardiomyopathy in mice. *Circ Arrhythm Electrophysiol* 8: 921–932, 2015. doi:10.1161/CIRCEP.115.001668.
567. **Lange A, Wickström SA, Jakobson M, Zent R, Sainio K, Fässler R.** Integrin-linked kinase is an adaptor with essential functions during mouse development. *Nature* 461: 1002–1006, 2009. doi:10.1038/nature08468.
568. **Attwell S, Mills J, Troussard A, Wu C, Dedhar S.** Integration of cell attachment, cytoskeletal localization, and signaling by integrin-linked kinase (ILK), CH-ILKBP, and the tumor suppressor PTEN. *Mol Biol Cell* 14: 4813–4825, 2003. doi:10.1091/mbc.e03-05-0308.
569. **Traister A, Walsh M, Aafaqi S, Lu M, Dai X, Henkleman MR, Momen A, Zhou YQ, Husain M, Arab S, Piran S, Hannigan G, Coles JG.** Mutation in integrin-linked kinase (ILK^{R211A}) and heat-shock protein 70 comprise a broadly cardioprotective complex. *PLoS One* 8: e77331, 2013. doi:10.1371/journal.pone.0077331.
570. **Traister A, Li M, Aafaqi S, Lu M, Arab S, Radisic M, Gross G, Guido F, Sherret J, Verma S, Slorach C, Mertens L, Hui W, Roy A, Delgado-Olguín P, Hannigan G, Maynes JT, Coles JG.** Integrin-linked kinase mediates force transduction in cardiomyocytes by modulating SERCA2a/PLN function. *Nat Commun* 5: 4533, 2014. doi:10.1038/ncomms5533.
571. **Traister A, Aafaqi S, Masse S, Dai X, Li M, Hinek A, Nanthakumar K, Hannigan G, Coles JG.** ILK induces cardiomyogenesis in the human heart. *PLoS One* 7: e37802, 2012. doi:10.1371/journal.pone.0037802.
572. **Janin A, N'Guyen K, Habib G, Dauphin C, Chanavat V, Bouvagnet P, Eschalier R, Streichenberger N, Chevalier P, Millat G.** Truncating mutations on myofibrillar myopathies causing genes as prevalent molecular explanations on patients with dilated cardiomyopathy. *Clin Genet* 92: 616–623, 2017. doi:10.1111/cge.13043.

573. Verdonschot JA, Vanhoutte EK, Claes GR, Heldermaan van den Enden AT, Hoeijmakers JG, Hellebrekers DM, de Haan A, Christiaans I, Deprez RH, Boen HM, van Craenenbroeck EM, Loeys BL, Hoedemaekers YM, Marcelis C, Kempers M, Brusse E, van Waning JI, Baas AF, Dooijes D, Asselbergs FW, Barge-Schaapveld DQ, Koopman P, van den Wijngaard A, Heymans SR, Krapels IP, Brunner HG. A mutation update for the FLNC gene in myopathies and cardiomyopathies. *Hum Mutat* 41: 1091–1111, 2020. doi:10.1002/humu.24004.
574. Oz S, Yonath H, Visochyk L, Ofek E, Landa N, Reznik-Wolf H, Ortiz-Genga M, Monserrat L, Ben-Gal T, Goitein O, Beinart R, Glikson M, Freimark D, Pras E, Arad M, Nof E. Reduction in Filamin C transcript is associated with arrhythmogenic cardiomyopathy in Ashkenazi Jews. *Int J Cardiol* 317: 133–138, 2020. doi:10.1016/j.ijcard.2020.04.005.
575. Akhtar MM, Lorenzini M, Pavlou M, Ochoa JP, O'Mahony C, Restrepo-Cordoba MA; European Genetic Cardiomyopathies Initiative Investigators, et al. Association of left ventricular systolic dysfunction among carriers of truncating variants in filamin C with frequent ventricular arrhythmia and end-stage heart failure. *JAMA Cardiol* 6: 891, 2021. doi:10.1001/jamacardio.2021.1106.
576. Brun F, Gigli M, Graw SL, Judge DP, Merlo M, Murray B, Calkins H, Sinagra G, Taylor MR, Mestroni L, James CA. FLNC truncations cause arrhythmogenic right ventricular cardiomyopathy. *J Med Genet* 57: 254–257, 2020. doi:10.1136/jmedgenet-2019-106394.
577. Begay RL, Graw SL, Sinagra G, Asimaki A, Rowland TJ, Slavov DB, Gowan K, Jones KL, Brun F, Merlo M, Miani D, Sweet M, Devaraj K, Wartchow EP, Gigli M, Puggia I, Salcedo EE, Garrity DM, Ambardekar AV, Buttrick P, Reece TB, Bristow MR, Saffitz JE, Mestroni L, Taylor MR. Filamin C truncation mutations are associated with arrhythmogenic dilated cardiomyopathy and changes in the cell-cell adhesion structures. *JACC Clin Electrophysiol* 4: 504–514, 2018. doi:10.1016/j.jacep.2017.12.003.
578. Eden M, Frey N. Cardiac filaminopathies: illuminating the divergent role of filamin C mutations in human cardiomyopathy. *J Clin Med* 10: 577, 2021. doi:10.3390/jcm10040577.
579. Ortiz-Genga MF, Cuenca S, Dal Ferro M, Zorio E, Salgado-Aranda R, Climent V, et al. Truncating FLNC mutations are associated with high-risk dilated and arrhythmogenic cardiomyopathies. *J Am Coll Cardiol* 68: 2440–2451, 2016. doi:10.1016/j.jacc.2016.09.927.
580. Reinstein E, Gutierrez-Fernandez A, Tzur S, Bormans C, Marcu S, Tayeb-Fligelman E, Vinkler C, Raas-Rothschild A, Irge D, Landau M, Shohat M, Puente XS, Behar DM, Lopez-Otin C. Congenital dilated cardiomyopathy caused by biallelic mutations in filamin C. *Eur J Hum Genet* 24: 1792–1796, 2016. doi:10.1038/ejhg.2016.110.
581. Frasson MZ, Jaeger CP. Dilated cardiomyopathy: new variant in the Filamin-C gene. *Arq Bras Cardiol* 117: 16–18, 2021. doi:10.36660/abc.20200199.
582. Valdés-Mas R, Gutiérrez-Fernández A, Gómez J, Coto E, Astudillo A, Puente DA, Reguero JR, Álvarez V, Moris C, León D, Martín M, Puente XS, López-Otin C. Mutations in filamin C cause a new form of familial hypertrophic cardiomyopathy. *Nat Commun* 5: 5326, 2014. doi:10.1038/ncomms6326.
583. Schänzer A, Schumann E, Zengeler D, Gulatz L, Maroli G, Ahting U, Sprengel A, Gräf S, Hahn A, Jux C, Acker T, Fürst DO, Rupp S, Schuld J, van der Ven PF. The p.Ala2430Val mutation in filamin C causes a hypertrophic myofibrillar cardiomyopathy. *J Muscle Res Cell Motil* 42: 381–397, 2021. doi:10.1007/s10974-021-09601-1.
584. Qin X, Li P, Qu HQ, Liu Y, Xia Y, Chen S, Yang Y, Huang S, Wen P, Zhou X, Li X, Wang Y, Tian L, Hakonarson H, Wu Y, Zhuang J. FLNC and MYLK2 gene mutations in a Chinese family with different phenotypes of cardiomyopathy. *Int Heart J* 62: 127–134, 2021. doi:10.1536/ihj.20-351.
585. Tucker NR, McLellan MA, Hu D, Ye J, Parsons VA, Mills RW, Clauss S, Dolmatova E, Shea MA, Milan DJ, Scott NS, Lindsay M, Lubitz SA, Domian IJ, Stone JR, Lin H, Ellinor PT. Novel mutation in FLNC (Filamin C) causes familial restrictive cardiomyopathy. *Circ Cardiovasc Genet* 10: e001780, 2017. doi:10.1161/CIRCGENETICS.117.001780.
586. Gómez J, Lorca R, Reguero JR, Moris C, Martín M, Tranche S, Alonso B, Iglesias S, Alvarez V, Díaz-Molina B, Avanzas P, Coto E. Screening of the *Filamin C* gene in a large cohort of hypertrophic cardiomyopathy patients. *Circ Cardiovasc Genet* 10: e001584, 2017. doi:10.1161/CIRCGENETICS.116.001584.
587. Kley RA, Hellenbroich Y, van der Ven PF, Fürst DO, Huebner A, Bruchertseifer V, Peters SA, Heyer CM, Kirschner J, Schröder R, Fischer D, Müller K, Tolksdorf K, Eger K, Germing A, Brodherr T, Reum C, Walter MC, Lochmüller H, Ketelsen UP, Vorgerd M. Clinical and morphological phenotype of the filamin myopathy: a study of 31 German patients. *Brain* 130: 3250–3264, 2007. doi:10.1093/brain/awm271.
588. Vorgerd M, van der Ven PF, Bruchertseifer V, Löwe T, Kley RA, Schröder R, Lochmüller H, Himmel M, Koehler K, Fürst DO, Huebner A. A mutation in the dimerization domain of filamin c causes a novel type of autosomal dominant myofibrillar myopathy. *Am J Hum Genet* 77: 297–304, 2005. doi:10.1086/431959.
589. Fürst DO, Goldfarb LG, Kley RA, Vorgerd M, Olivé M, van der Ven PF. Filamin C-related myopathies: pathology and mechanisms. *Acta Neuropathol* 125: 33–46, 2013. doi:10.1007/s00401-012-1054-9.
590. Walsh R, Buchan R, Wilk A, John S, Felkin LE, Thomson KL, Chiaw TH, Loong CC, Pua CJ, Raphael C, Prasad S, Barton PJ, Funke B, Watkins H, Ware JS, Cook SA. Defining the genetic architecture of hypertrophic cardiomyopathy: re-evaluating the role of non-sarcomeric genes. *Eur Heart J* 38: 3461–3468, 2017. doi:10.1093/eurheartj/ehw603.
591. Cui H, Wang J, Zhang C, Wu G, Zhu C, Tang B, Zou Y, Huang X, Hui R, Song L, Wang S. Mutation profile of FLNC gene and its prognostic relevance in patients with hypertrophic cardiomyopathy. *Mol Genet Genomic Med* 6: 1104–1113, 2018. doi:10.1002/mgg3.488.
592. Hartwig JH, Stossel TP. Isolation and properties of actin, myosin, and a new actin-binding protein in rabbit alveolar macrophages. *J Biol Chem* 250: 5696–5705, 1975. doi:10.1016/S0021-9258(19)41235-0.
593. Stossel TP, Hartwig JH. Interactions between actin, myosin, and an actin-binding protein from rabbit alveolar macrophages. Alveolar macrophage myosin Mg-2 + -adenosine triphosphatase requires a cofactor for activation by actin. *J Biol Chem* 250: 5706–5712, 1975. doi:10.1016/S0021-9258(19)41236-2.
594. Razinia Z, Mäkelä T, Ylänne J, Calderwood DA. Filamins in mechanosensing and signaling. *Annu Rev Biophys* 41: 227–246, 2012. doi:10.1146/annurev-biophys-050511-102252.
595. Ohashi K, Oshima K, Tachikawa M, Morikawa N, Hashimoto Y, Ito M, Mori H, Kuribayashi T, Terasaki AG. Chicken gizzard filamin, retina filamin and cgABP260 are respectively, smooth muscle-, non-muscle- and pan-muscle-type isoforms: distribution and localization in muscles. *Cell Motil Cytoskeleton* 61: 214–225, 2005. doi:10.1002/cm.20073.
596. Thompson TG, Chan YM, Hack AA, Brosius M, Rajala M, Lidov HG, McNally EM, Watkins S, Kunkel LM. Filamin 2 (FLN2): a muscle-specific sarcoglycan interacting protein. *J Cell Biol* 148: 115–126, 2000. doi:10.1083/jcb.148.1.115.
597. Popowicz GM, Schleicher M, Noegel AA, Holak TA. Filamins: promiscuous organizers of the cytoskeleton. *Trends Biochem Sci* 31: 411–419, 2006. doi:10.1016/j.tibs.2006.05.006.
598. Pudas R, Kiema TR, Butler PJ, Stewart M, Ylänne J. Structural basis for vertebrate filamin dimerization. *Structure* 13: 111–119, 2005. doi:10.1016/j.str.2004.10.014.
599. Himmel M, Van Der Ven PF, Stöcklein W, Fürst DO. The limits of promiscuity: isoform-specific dimerization of filamins. *Biochemistry* 42: 430–439, 2003. doi:10.1021/bi026501+.
600. van der Ven PF, Obermann WM, Lemke B, Gautel M, Weber K, Fürst DO. Characterization of muscle filamin isoforms suggests a possible role of gamma-filamin/ABP-L in sarcomeric Z-disc formation. *Cell Motil Cytoskeleton* 45: 149–162, 2000. doi:10.1002/(SICI)1097-0169(200002)45:2<149::AID-CM6>3.0.CO;2-G.
601. van der Ven PF, Wiesner S, Salmikangas P, Auerbach D, Himmel M, Kempa S, Hayess K, Pacholsky D, Taivainen A, Schröder R, Carpen O, Fürst DO. Indications for a novel muscular dystrophy pathway. gamma-filamin, the muscle-specific filamin isoform, interacts with myotilin. *J Cell Biol* 151: 235–248, 2000. doi:10.1083/jcb.151.2.235.
602. Gontier Y, Taivainen A, Fontao L, Sonnenberg A, van der Flier A, Carpen O, Faulkner G, Borradori L. The Z-disc proteins myotilin and FATZ-1 interact with each other and are connected to the sarcolemma via muscle-specific filamins. *J Cell Sci* 118: 3739–3749, 2005. doi:10.1242/jcs.02484.
603. Takada F, Vander Woude DL, Tong HQ, Thompson TG, Watkins SC, Kunkel LM, Beggs AH. Myozenin: an alpha-actinin- and gamma-

- filamin-binding protein of skeletal muscle Z-lines. *Proc Natl Acad Sci USA* 98: 1595–1600, 2001. doi:10.1073/pnas.041609698.
604. **Faulkner G, Pallavicini A, Comelli A, Salamon M, Bortoletto G, Ievolella C, Trevisan S, Kojic S, Dalla Vecchia F, Laveder P, Valle G, Lanfranchi G.** FATZ, a filamin-, actinin-, and telethonin-binding protein of the Z-disc of skeletal muscle. *J Biol Chem* 275: 41234–41242, 2000. doi:10.1074/jbc.M007493200.
605. **Linnemann A, van der Ven PF, Vakeel P, Albinus B, Simonis D, Bendas G, Schenk JA, Micheel B, Kley RA, Fürst DO.** The sarcomeric Z-disc component myopodin is a multiadapter protein that interacts with filamin and alpha-actinin. *Eur J Cell Biol* 89: 681–692, 2010. doi:10.1016/j.ejcb.2010.04.004.
606. **Molt S, Bührdel JB, Yakovlev S, Schein P, Orfanos Z, Kirfel G, Winter L, Wiche G, van der Ven PF, Rottbauer W, Just S, Belkin AM, Fürst DO.** Aciculin interacts with filamin C and Xin and is essential for myofibril assembly, remodeling and maintenance. *J Cell Sci* 127: 3578–3592, 2014. doi:10.1242/jcs.152157.
607. **Loo DT, Kanner SB, Aruffo A.** Filamin binds to the cytoplasmic domain of the beta1-integrin. Identification of amino acids responsible for this interaction. *J Biol Chem* 273: 23304–23312, 1998. doi:10.1074/jbc.273.36.23304.
608. **Zhang M, Liu J, Cheng A, Deyoung SM, Saltiel AR.** Identification of CAP as a costameric protein that interacts with filamin C. *Mol Biol Cell* 18: 4731–4740, 2007. doi:10.1091/mbc.e07-06-0628.
609. **Lu S, Carroll SL, Herrera AH, Ozanne B, Horowitz R.** New N-RAP-binding partners alpha-actinin, filamin and Krp1 detected by yeast two-hybrid screening: implications for myofibril assembly. *J Cell Sci* 116: 2169–2178, 2003. doi:10.1242/jcs.00425.
610. **van der Ven PF, Ehler E, Vakeel P, Eulitz S, Schenk JA, Milting H, Micheel B, Fürst DO.** Unusual splicing events result in distinct Xin isoforms that associate differentially with filamin c and Mena/VASP. *Exp Cell Res* 312: 2154–2167, 2006. doi:10.1016/j.yexcr.2006.03.015.
611. **Wadmore K, Azad AJ, Gehmlich K.** The Role of Z-disc Proteins in Myopathy and Cardiomyopathy. *Int J Mol Sci* 22: 3058, 2021. doi:10.3390/ijms22063058.
612. **Ehsan M, Jiang H, Thomson KL, Gehmlich K.** When signalling goes wrong: pathogenic variants in structural and signalling proteins causing cardiomyopathies. *J Muscle Res Cell Motil* 38: 303–316, 2017. doi:10.1007/s10974-017-9487-3.
613. **Dalkilic I, Schienda J, Thompson TG, Kunkel LM.** Loss of FilaminC (FLNC) results in severe defects in myogenesis and myotube structure. *Mol Cell Biol* 26: 6522–6534, 2006. doi:10.1128/MCB.00243-06.
614. **Zhou Y, Chen Z, Zhang L, Zhu M, Tan C, Zhou X, Evans SM, Fang X, Feng W, Chen J.** Loss of filamin C is catastrophic for heart function. *Circulation* 141: 869–871, 2020. doi:10.1161/CIRCULATIONAHA.119.044061.
615. **Primeau JO, Armanious GP, Fisher ME, Young HS.** The sarcoendoplasmic reticulum calcium ATPase. *Subcell Biochem* 87: 229–258, 2018. doi:10.1007/978-981-10-7757-9_8.
616. **Wiekliński MJ, Kannankeril PJ, Knollmann BC.** Molecular and tissue mechanisms of catecholaminergic polymorphic ventricular tachycardia. *J Physiol* 598: 2817–2834, 2020. doi:10.1113/JP276757.
617. **Rossi AE, Dirksen RT.** Sarcoplasmic reticulum: the dynamic calcium governor of muscle. *Muscle Nerve* 33: 715–731, 2006. doi:10.1002/mus.20512.
618. **Roston TM, Guo W, Krahn AD, Wang R, Van Petegem F, Sanatani S, Chen SR, Lehman A.** A novel RYR2 loss-of-function mutation (I4855M) is associated with left ventricular non-compaction and atypical catecholaminergic polymorphic ventricular tachycardia. *J Electrocardiol* 50: 227–233, 2017. doi:10.1016/j.jelectrocard.2016.09.006.
619. **Roux-Buisson N, Gandjbakhch E, Donal E, Probst V, Deharo JC, Chevalier P, Klug D, Mansencal N, Delacretaz E, Cosnay P, Scanu P, Extramiana F, Keller D, Hidden-Lucet F, Trapani J, Fouret P, Frank R, Fressart V, Fauré J, Lunardi J, Charron P.** Prevalence and significance of rare RYR2 variants in arrhythmogenic right ventricular cardiomyopathy/dysplasia: results of a systematic screening. *Heart Rhythm* 11: 1999–2009, 2014. doi:10.1016/j.hrthm.2014.07.020.
620. **Tiso N, Stephan DA, Nava A, Bagattin A, Devaney JM, Stanchi F, Larderet G, Brahmabhatt B, Brown K, Bauce B, Muriago M, Basso C, Thiene G, Danieli GA, Rampazzo A.** Identification of mutations in the cardiac ryanodine receptor gene in families affected with arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2). *Hum Mol Genet* 10: 189–194, 2001. doi:10.1093/hmg/10.3.189.
621. **Costa S, Medeiros-Domingo A, Gasperetti A, Breitenstein A, Steffel J, Guidetti F, Flammer A, Odening K, Ruschitzka F, Duru F, Saguner AM.** Familial dilated cardiomyopathy associated with a novel heterozygous RYR2 early truncating variant. *Cardiol J* 28: 173–175, 2021. doi:10.5603/CJ.a2020.0099.
622. **Sun B, Yao J, Ni M, Wei J, Zhong X, Guo W, et al.** Cardiac ryanodine receptor calcium release deficiency syndrome. *Sci Transl Med* 13: eaba7287, 2021. doi:10.1126/scitranslmed.aba7287.
623. **Walsh R, Offerhaus JA, Tadros R, Bezzina CR.** Minor hypertrophic cardiomyopathy genes, major insights into the genetics of cardiomyopathies. *Nat Rev Cardiol* (2021). doi:10.1038/s41569-021-00608-2.
624. **Zhang Q, Chen J, Qin Y, Wang J, Zhou L.** Mutations in voltage-gated L-type calcium channel: implications in cardiac arrhythmia. *Channels (Austin)* 12: 201–218, 2018. doi:10.1080/19336950.2018.1499368.
625. **Woon MT, Long PA, Reilly L, Evans JM, Keefe AM, Lea MR, Beglinger CJ, Balijepalli RC, Lee Y, Olson TM, Kamp TJ.** Pediatric dilated cardiomyopathy-associated LRRC10 (Leucine-Rich Repeat-Containing 10) variant reveals LRRC10 as an auxiliary subunit of cardiac L-type Ca²⁺ channels. *J Am Heart Assoc* 7: e006428, 2018. doi:10.1161/JAHA.117.006428.
626. **Qu XK, Yuan F, Li RG, Xu L, Jing WF, Liu H, Xu YJ, Zhang M, Liu X, Fang WY, Yang YQ, Qiu XB.** Prevalence and spectrum of LRRC10 mutations associated with idiopathic dilated cardiomyopathy. *Mol Med Rep* 12: 3718–3724, 2015. doi:10.3892/mmr.2015.3843.
627. **Vanninen SU, Leivo K, Seppälä EH, Aalto-Setälä K, Pitkänen O, Suursalmi P, Annala AP, Anttila I, Alastalo TP, Myllykangas S, Heliö TM, Koskenvuo JW.** Heterozygous junctophilin-2 (JPH2) p.(Thr161Lys) is a monogenic cause for HCM with heart failure. *PLoS One* 13: e0203422, 2018. doi:10.1371/journal.pone.0203422.
628. **Quick AP, Landstrom AP, Wang Q, Beavers DL, Reynolds JO, Barreto-Torres G, Tran V, Showell J, Philippen LE, Morris SA, Skapura D, Bos JM, Pedersen SE, Pautler RG, Ackerman MJ, Wehrens XH.** Novel junctophilin-2 mutation A405S is associated with basal septal hypertrophy and diastolic dysfunction. *JACC Basic Transl Sci* 2: 56–67, 2017. doi:10.1016/j.jacbs.2016.11.004.
629. **Matsushita Y, Furukawa T, Kasanuki H, Nishibatake M, Kurihara Y, Ikeda A, Kamatani N, Takeshima H, Matsuoka R.** Mutation of junctophilin type 2 associated with hypertrophic cardiomyopathy. *J Hum Genet* 52: 543–548, 2007. doi:10.1007/s10038-007-0149-y.
630. **Landstrom AP, Weisleder N, Bataiden KB, Bos JM, Tester DJ, Ommen SR, Wehrens XH, Claycomb WC, Ko JK, Hwang M, Pan Z, Ma J, Ackerman MJ.** Mutations in JPH2-encoded junctophilin-2 associated with hypertrophic cardiomyopathy in humans. *J Mol Cell Cardiol* 42: 1026–1035, 2007. doi:10.1016/j.yjmcc.2007.04.006.
631. **Jones EG, Mazaheri N, Maroofian R, Zamani M, Seifi T, Sedaghat A, Shariati G, Jamshidi Y, Allen HD, Wehrens XH, Galehdari H, Landstrom AP.** Analysis of enriched rare variants in JPH2-encoded junctophilin-2 among Greater Middle Eastern individuals reveals a novel homozygous variant associated with neonatal dilated cardiomyopathy. *Sci Rep* 9: 9038, 2019. doi:10.1038/s41598-019-44987-6.
632. **Garbino A, van Oort RJ, Dixit SS, Landstrom AP, Ackerman MJ, Wehrens XH.** Molecular evolution of the junctophilin gene family. *Physiol Genomics* 37: 175–186, 2009. doi:10.1152/physiolgenomics.00017.2009.
633. **Nishi M, Mizushima A, Nakagawara K, Takeshima H.** Characterization of human junctophilin subtype genes. *Biochem Biophys Res Commun* 273: 920–927, 2000. doi:10.1006/bbrc.2000.3011.
634. **Guo A, Wang Y, Chen B, Wang Y, Yuan J, Zhang L, Hall D, Wu J, Shi Y, Zhu Q, Chen C, Thiel WH, Zhan X, Weiss RM, Zhan F, Musselman CA, Pufall M, Zhu W, Au KF, Hong J, Anderson ME, Grueter CE, Song LS.** E-C coupling structural protein junctophilin-2 encodes a stress-adaptive transcription regulator. *Science* 362: eaan3303, 2018. doi:10.1126/science.aan3303.
635. **Eisner DA, Caldwell JL, Kistamáas K, Trafford AW.** Calcium and Excitation-Contraction Coupling in the Heart. *Circ Res* 121: 181–195, 2017. doi:10.1161/CIRCRESAHA.117.310230.
636. **Im YJ, Davis AJ, Perera IY, Johannes E, Allen NS, Boss WF.** The N-terminal membrane occupation and recognition nexus domain of Arabidopsis phosphatidylinositol phosphate kinase 1 regulates

- enzyme activity. *J Biol Chem* 282: 5443–5452, 2007. doi:10.1074/jbc.M611342200.
637. Komazaki S, Nakamura H. Functional analysis of mammalian genes using amphibian embryonic cells. *J Electron Microscop (Tokyo)* 53: 87–92, 2004. doi:10.1093/jmicro/53.1.87.
638. Takeshima H, Komazaki S, Nishi M, Iino M, Kangawa K. Junctophilins: a novel family of junctional membrane complex proteins. *Mol Cell* 6: 11–22, 2000. doi:10.1016/S1097-2765(00)00003-4.
639. Beavers DL, Wang W, Ather S, Voigt N, Garbino A, Dixit SS, Landstrom AP, Li N, Wang Q, Olivetto I, Dobrev D, Ackerman MJ, Wehrens XH. Mutation E169K in junctophilin-2 causes atrial fibrillation due to impaired RyR2 stabilization. *J Am Coll Cardiol* 62: 2010–2019, 2013. doi:10.1016/j.jacc.2013.06.052.
640. van Oort RJ, Garbino A, Wang W, Dixit SS, Landstrom AP, Gaur N, De Almeida AC, Skapura DG, Rudy Y, Burns AR, Ackerman MJ, Wehrens XH. Disrupted junctional membrane complexes and hyperactive ryanodine receptors after acute junctophilin knockdown in mice. *Circulation* 123: 979–988, 2011. doi:10.1161/CIRCULATIONAHA.110.006437.
641. Gross P, Johnson J, Romero CM, Eaton DM, Poulet C, Sanchez-Alonso J, Lucarelli C, Ross J, Gibb AA, Garbincius JF, Lambert J, Varol E, Yang Y, Wallner M, Feldsott EA, Kubo H, Berretta RM, Yu D, Rizzo V, Elrod J, Sabri A, Gorelik J, Chen X, Houser SR. Interaction of the joining region in junctophilin-2 with the L-type Ca^{2+} channel is pivotal for cardiac dyad assembly and intracellular Ca^{2+} dynamics. *Circ Res* 128: 92–114, 2021. doi:10.1161/CIRCRESAHA.119.315715.
642. Poulet C, Sanchez-Alonso J, Swiatlowska P, Mouy F, Lucarelli C, Alvarez-Laviada A, Gross P, Terracciano C, Houser S, Gorelik J. Junctophilin-2 tethers T-tubules and recruits functional L-type calcium channels to lipid rafts in adult cardiomyocytes. *Cardiovasc Res* 117: 149–161, 2021. doi:10.1093/cvr/cvaa033.
643. Jiang M, Zhang M, Howren M, Wang Y, Tan A, Balijepalli RC, Huizar JF, Tseng GN. JPH-2 interacts with Cai-handling proteins and ion channels in dyads: contribution to premature ventricular contraction-induced cardiomyopathy. *Heart Rhythm* 13: 743–752, 2016. doi:10.1016/j.hrthm.2015.10.037.
644. Fan HK, Luo TX, Zhao WD, Mu YH, Yang Y, Guo WJ, Tu HY, Zhang Q. Functional interaction of Junctophilin 2 with small-conductance Ca^{2+} -activated potassium channel subtype 2 (SK2) in mouse cardiac myocytes. *Acta Physiol* 222: e12986, 2018. doi:10.1111/apha.12986.
645. Liu C, Spinozzi S, Chen JY, Fang X, Feng W, Perkins G, Cattaneo P, Guimarães-Camboia N, Dalton ND, Peterson KL, Wu T, Ouyang K, Fu XD, Evans SM, Chen J. Nexilin is a new component of junctional membrane complexes required for cardiac t-tubule formation. *Circulation* 140: 55–66, 2019. doi:10.1161/CIRCULATIONAHA.119.039751.
646. Minamisawa S, Oshikawa J, Takeshima H, Hoshijima M, Wang Y, Chien KR, Ishikawa Y, Matsuoka R. Junctophilin type 2 is associated with caveolin-3 and is down-regulated in the hypertrophic and dilated cardiomyopathies. *Biochem Biophys Res Commun* 325: 852–856, 2004. doi:10.1016/j.bbrc.2004.10.107.
647. Quick AP, Wang Q, Philippen LE, Barreto-Torres G, Chiang DY, Beavers D, Wang G, Khalid M, Reynolds JO, Campbell HM, Showell J, McCauley MD, Scholten A, Wehrens XH. SPEG (striated muscle preferentially expressed protein kinase) is essential for cardiac function by regulating junctional membrane complex activity. *Circ Res* 120: 110–119, 2017. doi:10.1161/CIRCRESAHA.116.309977.
648. Reynolds JO, Chiang DY, Wang W, Beavers DL, Dixit SS, Skapura DG, Landstrom AP, Song LS, Ackerman MJ, Wehrens XH. Junctophilin-2 is necessary for T-tubule maturation during mouse heart development. *Cardiovasc Res* 100: 44–53, 2013. doi:10.1093/cvr/cvt133.
649. Chen B, Guo A, Zhang C, Chen R, Zhu Y, Hong J, Kutschke W, Zimmerman K, Weiss RM, Zingman L, Anderson ME, Wehrens XH, Song LS. Critical roles of junctophilin-2 in T-tubule and excitation-contraction coupling maturation during postnatal development. *Cardiovasc Res* 100: 54–62, 2013. doi:10.1093/cvr/cvt180.
650. Brandenburg S, Pawlowitz J, Eikenbusch B, Peper J, Kohl T, Mitronova GY, Sossalla S, Hasenfuss G, Wehrens XH, Kohl P, Rog-Zielinska EA, Lehnart SE. Junctophilin-2 expression rescues atrial dysfunction through polyadic junctional membrane complex biogenesis. *JCI Insight* 4: e127116, 2019. doi:10.1172/jci.insight.127116.
651. Wang W, Landstrom AP, Wang Q, Munro ML, Beavers D, Ackerman MJ, Soeller C, Wehrens XH. Reduced junctional Na^{+}/Ca^{2+} -exchanger activity contributes to sarcoplasmic reticulum Ca^{2+} leak in junctophilin-2-deficient mice. *Am J Physiol Heart Circ Physiol* 307: H1317–H1326, 2014. [Erratum in *Am J Physiol Heart Circ Physiol* 314: H1115, 2018]. doi:10.1152/ajpheart.00413.2014.
652. Feng W, Liu C, Spinozzi S, Wang L, Evans SM, Chen J. Identifying the cardiac dyad proteome in vivo by a BiolD2 knock-in strategy. *Circulation* 141: 940–942, 2020. doi:10.1161/CIRCULATIONAHA.119.043434.
653. Gingras AC, Abe KT, Raught B. Getting to know the neighborhood: using proximity-dependent biotinylation to characterize protein complexes and map organelles. *Curr Opin Chem Biol* 48: 44–54, 2019. doi:10.1016/j.cbpa.2018.10.017.
654. Wang H, Li Z, Wang J, Sun K, Cui Q, Song L, Zou Y, Wang X, Liu X, Hui R, Fan Y. Mutations in NEXN, a Z-disc gene, are associated with hypertrophic cardiomyopathy. *Am J Hum Genet* 87: 687–693, 2010. doi:10.1016/j.ajhg.2010.10.002.
655. Hassel D, Dahme T, Erdmann J, Meder B, Hüge A, Stoll M, Just S, Hess A, Ehlermann P, Weichenhan D, Grimmmer M, Liptau H, Hetzer R, Regitz-Zagrosek V, Fischer C, Nurnberg P, Schunkert H, Katus HA, Rottbauer W. Nexilin mutations destabilize cardiac Z-discs and lead to dilated cardiomyopathy. *Nat Med* 15: 1281–1288, 2009. doi:10.1038/nm.2037.
656. Bruyndonckx L, Vogelzang JL, Bugiani M, Straver B, Kuipers IM, Onland W, Nannenber EA, Clur SA, van der Crabben SN. Childhood onset nexilin dilated cardiomyopathy: a heterozygous and a homozygous case. *Am J Med Genet A* 185: 2464–2470, 2021. doi:10.1002/ajmg.a.62231.
657. Ohtsuka T, Nakanishi H, Ikeda W, Satoh A, Momose Y, Nishioka H, Takai Y. Nexilin: a novel actin filament-binding protein localized at cell-matrix adherens junction. *J Cell Biol* 143: 1227–1238, 1998. doi:10.1083/jcb.143.5.1227.
658. Zhu B, Rippe C, Holmberg J, Zeng S, Perisic L, Albinsson S, Hedin U, Uvelius B, Sward K. Nexilin/NEXN controls actin polymerization in smooth muscle and is regulated by myocardium family coactivators and YAP. *Sci Rep* 8: 13025, 2018. doi:10.1038/s41598-018-31328-2.
659. Hu YP, Guo FX, Xu YJ, Li P, Lu ZF, McVey DG, Zheng L, Wang Q, Ye JH, Kang CM, Wu SG, Zhao JJ, Ma X, Yang Z, Fang FC, Qiu YR, Xu BM, Xiao L, Wu Q, Wu LM, Ding L, Webb TR, Samani NJ, Ye S. Long noncoding RNA NEXN-AS1 mitigates atherosclerosis by regulating the actin-binding protein NEXN. *J Clin Invest* 129: 1115–1128, 2019. doi:10.1172/JCI98230.
660. Aherrahrou Z, Schlossarek S, Stoelting S, Klinger M, Geertz B, Weinberger F, Kessler T, Aherrahrou R, Moreth K, Bekeredjian R, Hrabec de Angelis M, Just S, Rottbauer W, Eschenhagen T, Schunkert H, Carrier L, Erdmann J. Knock-out of nexilin in mice leads to dilated cardiomyopathy and endomyocardial fibroelastosis. *Basic Res Cardiol* 111: 6, 2016. doi:10.1007/s00395-015-0522-5.
661. Spinozzi S, Liu C, Chen Z, Feng W, Zhang L, Ouyang K, Evans SM, Chen J. Nexilin is necessary for maintaining the transverse-axial tubular system in adult cardiomyocytes. *Circ Heart Fail* 13: e006935, 2020. doi:10.1161/CIRCHEARTFAILURE.120.006935.
662. Liu C, Spinozzi S, Feng W, Chen Z, Zhang L, Zhu S, Wu T, Fang X, Ouyang K, Evans SM, Chen J. Homozygous G650del nexilin variant causes cardiomyopathy in mice. *JCI Insight* 5: e138780, 2020. doi:10.1172/jci.insight.138780.
663. Stroud MJ, Banerjee I, Veervers J, Chen J. Linker of nucleoskeleton and cytoskeleton complex proteins in cardiac structure, function, and disease. *Circ Res* 114: 538–548, 2014. doi:10.1161/CIRCRESAHA.114.301236.
664. Stroud MJ. Linker of nucleoskeleton and cytoskeleton complex proteins in cardiomyopathy. *Biophys Rev* 10: 1033–1051, 2018. doi:10.1007/s12551-018-0431-6.
665. Ross JA, Stroud MJ. The nucleus: mechanosensing in cardiac disease. *Int J Biochem Cell Biol* 137: 106035, 2021. doi:10.1016/j.biocel.2021.106035.
666. Piccus R, Brayson D. The nuclear envelope: LINCing tissue mechanics to genome regulation in cardiac and skeletal muscle. *Biol Lett* 16: 20200302, 2020. doi:10.1098/rsbl.2020.0302.
667. Kohli S, Ahuja S, Rani V. Transcription factors in heart: promising therapeutic targets in cardiac hypertrophy. *Curr Cardiol Rev* 7: 262–271, 2011. doi:10.2174/157340311799960618.

668. **Mikhailov AT, Torrado M.** Myocardial transcription factors in diastolic dysfunction: clues for model systems and disease. *Heart Fail Rev* 21: 783–794, 2016. doi:10.1007/s10741-016-9569-0.
669. **Williams K, Carson J, Lo C.** Genetics of congenital heart disease. *Biomolecules* 9: 879, 2019. doi:10.3390/biom9120879.
670. **Bruneau BG.** Signaling and transcriptional networks in heart development and regeneration. *Cold Spring Harb Perspect Biol* 5: a008292, 2013. doi:10.1101/cshperspect.a008292.
671. **Heller SA, Shih R, Kalra R, Kang PB.** Emery-Dreifuss muscular dystrophy. *Muscle Nerve* 61: 436–448, 2020. doi:10.1002/mus.26782.
672. **Janin A, Gache V.** Nesprins and lamins in health and diseases of cardiac and skeletal muscles. *Front Physiol* 9: 1277, 2018. doi:10.3389/fphys.2018.01277.
673. **Boriani G, Biagini E, Ziacchi M, Malavasi VL, Vitolo M, Talarico M, Mauro E, Gorlato G, Lattanzi G.** Cardiomyopathies from bench to bedside: challenges in clinical decision-making with focus on arrhythmia-related outcomes. *Nucleus* 9: 442–459, 2018. doi:10.1080/19491034.2018.1506680.
674. **Meinke P, Mattioli E, Haque F, Antoku S, Columbaro M, Straatman KR, Worman HJ, Gundersen GG, Lattanzi G, Wehnert M, Shackleton S.** Muscular dystrophy-associated SUN1 and SUN2 variants disrupt nuclear-cytoskeletal connections and myonuclear organization. *PLoS Genet* 10: e1004605, 2014. doi:10.1371/journal.pgen.1004605.
675. **Boone PM, Yuan B, Gu S, Ma Z, Gambin T, Gonzaga-Jauregui C, Jain M, Murdock TJ, White JJ, Jhangiani SN, Walker K, Wang Q, Muzny DM, Gibbs RA, Hejtmanec JF, Lupski JR, Posey JE, Lewis RA.** Hutterite-type cataract maps to chromosome 6p21.32–p21.31, cosegregates with a homozygous mutation in LEMD2, and is associated with sudden cardiac death. *Mol Genet Genomic Med* 4: 77–94, 2016. doi:10.1002/mggg.3.181.
676. **Abdelfatah N, Chen R, Duff HJ, Seifer CM, Buffo I, Huculak C, Clarke S, Clegg R, Jassal DS, Gordon PM, Ober C, Frosk P, Gerull B; Care4Rare Canada Consortium.** Characterization of a unique form of arrhythmic cardiomyopathy caused by recessive mutation in LEMD2. *JACC Basic Transl Sci* 4: 204–221, 2019. doi:10.1016/j.jacbs.2018.12.001.
677. **Dorboz I, Coutelier M, Bertrand AT, Caberg JH, Elmaleh-Berges M, Laine J, Stevanin G, Bonne G, Boespflug-Tanguy O, Servais L.** Severe dystonia, cerebellar atrophy, and cardiomyopathy likely caused by a missense mutation in TOR1AIP1. *Orphanet J Rare Dis* 9: 174, 2014. doi:10.1186/s13023-014-0174-9.
678. **Kayman-Kurekci G, Talim B, Korkusuz P, Sayar N, Sarioglu T, Oncel I, Sharafi P, Gundesli H, Balci-Hayta B, Purali N, Serdaroglu-Ofazer P, Topaloglu H, Dincer P.** Mutation in TOR1AIP1 encoding LAPIB in a form of muscular dystrophy: a novel gene related to nuclear envelopathies. *Neuromuscul Disord* 24: 624–633, 2014. doi:10.1016/j.nmd.2014.04.007.
679. **Taylor MR, Slavov D, Gajewski A, Vicek S, Ku L, Fain PR, Carniel E, Di Lenarda A, Sinagra G, Boucek MM, Cavanaugh J, Graw SL, Ruegg P, Feiger J, Zhu X, Ferguson DA, Bristow MR, Gotzmann J, Foisner R, Mestroni L; Familial Cardiomyopathy Registry Research Group.** Thymopoietin (lamina-associated polypeptide 2) gene mutation associated with dilated cardiomyopathy. *Hum Mutat* 26: 566–574, 2005. doi:10.1002/humu.20250.
680. **Haskell GT, Jensen BC, Samsa LA, Marchuk D, Huang W, Skrzynia C, Tilley C, Seifert BA, Rivera-Muñoz EA, Koller B, Wilhelmsen KC, Liu J, Alhosaini H, Weck KE, Evans JP, Berg JS.** Whole exome sequencing identifies truncating variants in nuclear envelope genes in patients with cardiovascular disease. *Circ Cardiovasc Genet* 10: e001443, 2017. doi:10.1161/CIRCGENETICS.116.001443.
681. **Zhang X, Chen S, Yoo S, Chakrabarti S, Zhang T, Ke T, Oberti C, Yong SL, Fang F, Li L, de la Fuente R, Wang L, Chen Q, Wang QK.** Mutation in nuclear pore component NUP155 leads to atrial fibrillation and early sudden cardiac death. *Cell* 135: 1017–1027, 2008. doi:10.1016/j.cell.2008.10.022.
682. **Kölbel H, Abicht A, Schwartz O, Katona I, Paulus W, Neuen-Jacob E, Weis J, Schara U.** Characteristic clinical and ultrastructural findings in nesprinopathies. *Eur J Paediatr Neurol* 23: 254–261, 2019. doi:10.1016/j.ejpn.2018.12.011.
683. **Puckelwartz MJ, Kessler EJ, Kim G, Dewitt MM, Zhang Y, Earley JU, Depreux FF, Holaska J, Mewborn SK, Pytel P, McNally EM.** Nesprin-1 mutations in human and murine cardiomyopathy. *J Mol Cell Cardiol* 48: 600–608, 2010. doi:10.1016/j.yjmcc.2009.11.006.
684. **Szabadosova V, Boronova I, Ferenc P, Tothova I, Bernasovska J, Zigova M, Kmec J, Bernasovsky I.** Analysis of selected genes associated with cardiomyopathy by next-generation sequencing. *J Clin Lab Anal* 32: e22254, 2018. doi:10.1002/jcla.22254.
685. **Zhao T, Ma Y, Zhang Z, Xian J, Geng X, Wang F, Huang J, Yang Z, Luo Y, Lin Y.** Young and early-onset dilated cardiomyopathy with malignant ventricular arrhythmia and sudden cardiac death induced by the heterozygous LDB3, MYH6, and SYNE1 missense mutations. *Ann Noninvasive Electrocardiol* 26: e12840, 2021. doi:10.1111/anec.12840.
686. **Zhou C, Li C, Zhou B, Sun H, Koullourou V, Holt I, Puckelwartz MJ, Warren DT, Hayward R, Lin Z, Zhang L, Morris GE, McNally EM, Shackleton S, Rao L, Shanahan CM, Zhang Q.** Novel nesprin-1 mutations associated with dilated cardiomyopathy cause nuclear envelope disruption and defects in myogenesis. *Hum Mol Genet* 26: 2258–2276, 2017. doi:10.1093/hmg/ddx116.
687. **Zhang Q, Bethmann C, Worth NF, Davies JD, Wasner C, Feuer A, Ragnauth CD, Yi Q, Mellad JA, Warren DT, Wheeler MA, Ellis JA, Skepper JN, Vorgerd M, Schlotter-Weigel B, Weissberg PL, Roberts RG, Wehnert M, Shanahan CM.** Nesprin-1 and -2 are involved in the pathogenesis of Emery Dreifuss muscular dystrophy and are critical for nuclear envelope integrity. *Hum Mol Genet* 16: 2816–2833, 2007. doi:10.1093/hmg/ddm238.
688. **Sandra M, Maria Pia L, Stefano C, Pietro P, Crociani P, Aldo R, Giuseppe DS, Massimo C.** Emery-Dreifuss muscular dystrophy type 4: a new SYNE1 mutation associated with hypertrophic cardiomyopathy masked by a perinatal distal-repeated spastic diplegia. *Clin Case Rep* 7: 1078–1082, 2019. doi:10.1002/ccr3.2140.
689. **Indelicato E, Nachbauer W, Fauth C, Krabichler B, Schossig A, Eigentler A, Dichtl W, Wenning G, Wagner M, Fanciulli A, Janecke A, Boesch S.** SYNE1-ataxia: novel genotypic and phenotypic findings. *Parkinsonism Relat Disord* 62: 210–214, 2019. doi:10.1016/j.parkreldis.2018.12.007.
690. **Rajgor D, Mellad JA, Autore F, Zhang Q, Shanahan CM.** Multiple novel nesprin-1 and nesprin-2 variants act as versatile tissue-specific intracellular scaffolds. *PLoS One* 7: e40098, 2012. doi:10.1371/journal.pone.0040098.
691. **Zhang Q, Ragnauth C, Greener MJ, Shanahan CM, Roberts RG.** The nesprins are giant actin-binding proteins, orthologous to *Drosophila melanogaster* muscle protein MSP-300. *Genomics* 80: 473–481, 2002. doi:10.1006/geno.2002.6859.
692. **Zhen YY, Libotte T, Munck M, Noegel AA, Korenbaum E.** NUANCE, a giant protein connecting the nucleus and actin cytoskeleton. *J Cell Sci* 115: 3207–3222, 2002. doi:10.1242/jcs.115.15.3207.
693. **Padmakumar VC, Abraham S, Braune S, Noegel AA, Tunggal B, Karakesisoglou I, Korenbaum E.** Enaptin, a giant actin-binding protein, is an element of the nuclear membrane and the actin cytoskeleton. *Exp Cell Res* 295: 330–339, 2004. doi:10.1016/j.yexcr.2004.01.014.
694. **Sosa BA, Rothbaler A, Kutay U, Schwartz TU.** LINC complexes form by binding of three KASH peptides to domain interfaces of trimeric SUN proteins. *Cell* 149: 1035–1047, 2012. doi:10.1016/j.cell.2012.03.046.
695. **Padmakumar VC, Libotte T, Lu W, Zaim H, Abraham S, Noegel AA, Gotzmann J, Foisner R, Karakesisoglou I.** The inner nuclear membrane protein Sun1 mediates the anchorage of Nesprin-2 to the nuclear envelope. *J Cell Sci* 118: 3419–3430, 2005. doi:10.1242/jcs.02471.
696. **Duong NT, Morris GE, Lam Le T, Zhang Q, Sewry CA, Shanahan CM, Holt I.** Nesprins: tissue-specific expression of epsilon and other short isoforms. *PLoS One* 9: e94380, 2014. doi:10.1371/journal.pone.0094380.
697. **Simpson JG, Roberts RG.** Patterns of evolutionary conservation in the nesprin genes highlight probable functionally important protein domains and isoforms. *Biochem Soc Trans* 36: 1359–1367, 2008. doi:10.1042/BST0361359.
698. **Zhang Q, Ragnauth CD, Skepper JN, Worth NF, Warren DT, Roberts RG, Weissberg PL, Ellis JA, Shanahan CM.** Nesprin-2 is a multi-isomeric protein that binds lamin and emerin at the nuclear envelope and forms a subcellular network in skeletal muscle. *J Cell Sci* 118: 673–687, 2005. doi:10.1242/jcs.01642.
699. **Haque F, Mazzeo D, Patel JT, Smallwood DT, Ellis JA, Shanahan CM, Shackleton S.** Mammalian SUN protein interaction networks at the inner nuclear membrane and their role in laminopathy disease

- processes. *J Biol Chem* 285: 3487–3498, 2010. doi:10.1074/jbc.M109.071910.
700. Yang L, Munck M, Swaminathan K, Kapinos LE, Noegel AA, Neumann S. Mutations in LMNA modulate the lamin A–Nesprin-2 interaction and cause LINC complex alterations. *PLoS One* 8: e71850, 2013. doi:10.1371/journal.pone.0071850.
701. Mislow JM, Holaska JM, Kim MS, Lee KK, Segura-Totten M, Wilson KL, McNally EM. Nesprin-1alpha self-associates and binds directly to emerin and lamin A in vitro. *FEBS Lett* 525: 135–140, 2002. doi:10.1016/S0014-5793(02)03105-8.
702. Mislow JM, Kim MS, Davis DB, McNally EM. Myne-1, a spectrin repeat transmembrane protein of the myocyte inner nuclear membrane, interacts with lamin A/C. *J Cell Sci* 115: 61–70, 2002. doi:10.1242/jcs.115.1.61.
703. Pare GC, Easlick JL, Mislow JM, McNally EM, Kapiloff MS. Nesprin-1alpha contributes to the targeting of mAKAP to the cardiac myocyte nuclear envelope. *Exp Cell Res* 303: 388–399, 2005. doi:10.1016/j.yexcr.2004.10.009.
704. Neumann S, Schneider M, Daugherty RL, Gottardi CJ, Eming SA, Beijer A, Noegel AA, Karakesisoglou I. Nesprin-2 interacts with alpha-catenin and regulates Wnt signaling at the nuclear envelope. *J Biol Chem* 285: 34932–34938, 2010. doi:10.1074/jbc.M110.119651.
705. Schneider M, Lu W, Neumann S, Brachner A, Gotzmann J, Noegel AA, Karakesisoglou I. Molecular mechanisms of centrosome and cytoskeleton anchorage at the nuclear envelope. *Cell Mol Life Sci* 68: 1593–1610, 2011. doi:10.1007/s00018-010-0535-z.
706. Wilson MH, Holzbaaur EL. Opposing microtubule motors drive robust nuclear dynamics in developing muscle cells. *J Cell Sci* 125: 4158–4169, 2012. doi:10.1242/jcs.108688.
707. Wilson MH, Holzbaaur EL. Nesprins anchor kinesin-1 motors to the nucleus to drive nuclear distribution in muscle cells. *Development* 142: 218–228, 2015. doi:10.1242/dev.114769.
708. Puckelwartz MJ, Kessler E, Zhang Y, Hodzic D, Randles KN, Morris G, Earley JU, Hadhazy M, Holaska JM, Mewborn SK, Pytel P, McNally EM. Disruption of nesprin-1 produces an Emery Dreifuss muscular dystrophy-like phenotype in mice. *Hum Mol Genet* 18: 607–620, 2009. doi:10.1093/hmg/ddn386.
709. Zhang X, Xu R, Zhu B, Yang X, Ding X, Duan S, Xu T, Zhuang Y, Han M. Syne-1 and Syne-2 play crucial roles in myonuclear anchorage and motor neuron innervation. *Development* 134: 901–908, 2007. doi:10.1242/dev.02783.
710. Zhang J, Felder A, Liu Y, Guo LT, Lange S, Dalton ND, Gu Y, Peterson KL, Mizisin AP, Shelton GD, Lieber RL, Chen J. Nesprin 1 is critical for nuclear positioning and anchorage. *Hum Mol Genet* 19: 329–341, 2010. doi:10.1093/hmg/ddp499.
711. Chapman MA, Zhang J, Banerjee I, Guo LT, Zhang Z, Shelton GD, Ouyang K, Lieber RL, Chen J. Disruption of both nesprin 1 and desmin results in nuclear anchorage defects and fibrosis in skeletal muscle. *Hum Mol Genet* 23: 5879–5892, 2014. doi:10.1093/hmg/ddu310.
712. Stroud MJ, Feng W, Zhang J, Veevers J, Fang X, Gerace L, Chen J. Nesprin 1alpha2 is essential for mouse postnatal viability and nuclear positioning in skeletal muscle. *J Cell Biol* 216: 1915–1924, 2017. doi:10.1083/jcb.201612128.
713. Banerjee I, Zhang J, Moore-Morris T, Pfeiffer E, Buchholz KS, Liu A, Ouyang K, Stroud MJ, Gerace L, Evans SM, McCulloch A, Chen J. Targeted ablation of nesprin 1 and nesprin 2 from murine myocardium results in cardiomyopathy, altered nuclear morphology and inhibition of the biomechanical gene response. *PLoS Genet* 10: e1004114, 2014. doi:10.1371/journal.pgen.1004114.
714. Mroß C, Marko M, Munck M, Glöckner G, Motameny S, Altmüller J, Noegel AA, Eichinger L, Peche VS, Neumann S. Depletion of Nesprin-2 is associated with an embryonic lethal phenotype in mice. *Nucleus* 9: 503–515, 2018. doi:10.1080/19491034.2018.1523664.
715. Dominguez F, Zorio E, Jimenez-Jaimez J, Salguero-Bodes R, Zwart R, Gonzalez-Lopez E, Molina P, Bermúdez-Jiménez F, Delgado JF, Braza-Boïls A, Bornstein B, Toquero J, Segovia J, Van Tintelen JP, Lara-Pezzi E, Garcia-Pavia P. Clinical characteristics and determinants of the phenotype in TMEM43 arrhythmogenic right ventricular cardiomyopathy type 5. *Heart Rhythm* 17: 945–954, 2020. doi:10.1016/j.hrthm.2020.01.035.
716. Baskin B, Skinner JR, Sanatani S, Terespolsky D, Krahn AD, Ray PN, Scherer SW, Hamilton RM. TMEM43 mutations associated with arrhythmogenic right ventricular cardiomyopathy in non-Newfoundland populations. *Hum Genet* 132: 1245–1252, 2013. doi:10.1007/s00439-013-1323-2.
717. Christensen AH, Andersen CB, Tybjaerg-Hansen A, Haunso S, Svendsen JH. Mutation analysis and evaluation of the cardiac localization of TMEM43 in arrhythmogenic right ventricular cardiomyopathy. *Clin Genet* 80: 256–264, 2011. doi:10.1111/j.1399-0004.2011.01623.x.
718. Mermer ND, Hodgkinson KA, Haywood AF, Connors S, French VM, Drenckhahn JD, Kupprion C, Ramadanova K, Thierfelder L, McKenna W, Gallagher B, Morris-Larkin L, Bassett AS, Parfrey PS, Young TL. Arrhythmogenic right ventricular cardiomyopathy type 5 is a fully penetrant, lethal arrhythmic disorder caused by a missense mutation in the TMEM43 gene. *Am J Hum Genet* 82: 809–821, 2008. doi:10.1016/j.ajhg.2008.01.010.
719. Haywood AF, Mermer ND, Hodgkinson KA, Houston J, Syrris P, Booth V, Connors S, Pantazis A, Quarta G, Elliott P, McKenna W, Young TL. Recurrent missense mutations in TMEM43 (ARVD5) due to founder effects cause arrhythmogenic cardiomyopathies in the UK and Canada. *Eur Heart J* 34: 1002–1011, 2013. doi:10.1093/eurheartj/ehs383.
720. Milting H, Klauke B, Christensen AH, Müsebeck J, Walhorn V, Grannemann S, Münnich T, Ščarić T, Rasmussen TB, Jensen HK, Mogensen J, Baecker C, Romaker E, Laser KT, zu Knyphausen E, Kassner A, Gummert J, Judge DP, Connors S, Hodgkinson K, Young TL, van der Zwaag PA, van Tintelen JP, Anselmetti D. The TMEM43 Newfoundland mutation p.S358L causing ARVC-5 was imported from Europe and increases the stiffness of the cell nucleus. *Eur Heart J* 36: 872–881, 2015. doi:10.1093/eurheartj/ehu077.
721. Honda T, Kanai Y, Ohno S, Ando H, Honda M, Niwano S, Ishii M. Fetal arrhythmogenic right ventricular cardiomyopathy with double mutations in TMEM43. *Pediatr Int* 58: 409–411, 2016. doi:10.1111/ped.12832.
722. Liang WC, Mitsuhashi H, Keduka E, Nonaka I, Noguchi S, Nishino I, Hayashi YK. TMEM43 mutations in Emery-Dreifuss muscular dystrophy-related myopathy. *Ann Neurol* 69: 1005–1013, 2011. doi:10.1002/ana.22338.
723. James CA, Jongbloed JD, Hershberger RE, Morales A, Judge DP, Syrris P, Pilichou K, Domingo AM, Murray B, Cadrin-Tourigny J, Deprez RL, Celeghin R, Protonotarios A, Asatryan B, Brown E, Jordan E, McGlaughon J, Thaxton C, Kurtz CL, van Tintelen JP. International evidence based reappraisal of genes associated with arrhythmogenic right ventricular cardiomyopathy using the Clinical Genome Resource Framework. *Circ Genom Precis Med* 14: e003273, 2021. doi:10.1161/CIRCGEN.120.003273.
724. Bengtsson L, Otto H. LUMA interacts with emerin and influences its distribution at the inner nuclear membrane. *J Cell Sci* 121: 536–548, 2008. doi:10.1242/jcs.019281.
725. Dreger M, Bengtsson L, Schöneberg T, Otto H, Hucho F. Nuclear envelope proteomics: novel integral membrane proteins of the inner nuclear membrane. *Proc Natl Acad Sci USA* 98: 11943–11948, 2001. doi:10.1073/pnas.211201898.
726. Padrón-Barthe L, Villalba-Otero M, Gómez-Salineró JM, Domínguez F, Román M, Larrasa-Alonso J, Ortiz-Sánchez P, Martínez F, Lopez-Olañeta M, Bonzon-Kulichenko E, Vazquez J, Martí-Gómez C, Santiago DJ, Prados B, Giovinazzo G, Gomez-Gavro MV, Priori S, Garcia-Pavia P, Lara-Pezzi E. Severe cardiac dysfunction and death caused by arrhythmogenic right ventricular cardiomyopathy type 5 are improved by inhibition of glycogen synthase kinase-3beta. *Circulation* 140: 1188–1204, 2019. doi:10.1161/CIRCULATIONAHA.119.040366.
727. Stroud MJ, Fang X, Zhang J, Guimarães-Camboa N, Veevers J, Dalton ND, Gu Y, Bradford WH, Peterson KL, Evans SM, Gerace L, Chen J. Luma is not essential for murine cardiac development and function. *Cardiovasc Res* 114: 378–388, 2018. doi:10.1093/cvr/cvx205.
728. Rouhi L, Cheedipudi SM, Chen SN, Fan S, Lombardi R, Chen X, Coarfa C, Robertson MJ, Gurha P, Marian AJ. Haplo-insufficiency of Tmem43 in cardiac myocytes activates the DNA damage response pathway leading to a late-onset senescence-associated pro-fibrotic cardiomyopathy. *Cardiovasc Res* 117: 2377–2394, 2021. doi:10.1093/cvr/cvaa300.
729. Zheng G, Jiang C, Li Y, Yang D, Ma Y, Zhang B, Li X, Zhang P, Hu X, Zhao X, Du J, Lin X. TMEM43-S358L mutation enhances NF-kappaB-TGFbeta signal cascade in arrhythmogenic right

- ventricular dysplasia/cardiomyopathy. *Protein Cell* 10: 104–119, 2019. doi:10.1007/s13238-018-0563-2.
730. Liu C, Shen A, Li X, Jiao W, Zhang X, Li Z. T-box transcription factor TBX20 mutations in Chinese patients with congenital heart disease. *Eur J Med Genet* 51: 580–587, 2008. doi:10.1016/j.ejmg.2008.09.001.
731. Kirk EP, Sunde M, Costa MW, Rankin SA, Wolstein O, Castro ML, Butler TL, Hyun C, Guo G, Otway R, Mackay JP, Waddell LB, Cole AD, Hayward C, Keogh A, Macdonald P, Griffiths L, Fatkin D, Sholler GF, Zorn AM, Feneley MP, Winlaw DS, Harvey RP. Mutations in cardiac T-box factor gene TBX20 are associated with diverse cardiac pathologies, including defects of septation and valvulogenesis and cardiomyopathy. *Am J Hum Genet* 81: 280–291, 2007. doi:10.1086/519530.
732. Posch MG, Gramlich M, Sunde M, Schmitt KR, Lee SH, Richter S, Kersten A, Perrot A, Panek AN, Al Khatib IH, Nemer G, Mégarbané A, Dietz R, Stiller B, Berger F, Harvey RP, Ozcelik C. A gain-of-function TBX20 mutation causes congenital atrial septal defects, patent foramen ovale and cardiac valve defects. *J Med Genet* 47: 230–235, 2010. doi:10.1136/jmg.2009.069997.
733. Zhao CM, Bing-Sun , Song HM, Wang J, Xu WJ, Jiang JF, Qiu XB, Yuan F, Xu JH, Yang YQ. TBX20 loss-of-function mutation associated with familial dilated cardiomyopathy. *Clin Chem Lab Med* 54: 325–332, 2016. doi:10.1515/ccml-2015-0328.
734. Zhou YM, Dai XY, Huang RT, Xue S, Xu YJ, Qiu XB, Yang YQ. A novel TBX20 loss-of-function mutation contributes to adult-onset dilated cardiomyopathy or congenital atrial septal defect. *Mol Med Rep* 14: 3307–3314, 2016. doi:10.3892/mmr.2016.5609.
735. Kodo K, Ong SG, Jahanbani F, Termglinchan V, Hirono K, InanlooRahatloo K, Ebert AD, Shukla P, Abilez OJ, Churko JM, Karakikes I, Jung G, Ichida F, Wu SM, Snyder MP, Bernstein D, Wu JC. iPSC-derived cardiomyocytes reveal abnormal TGF- β signalling in left ventricular non-compaction cardiomyopathy. *Nat Cell Biol* 18: 1031–1042, 2016. doi:10.1038/ncb3411.
736. Mittal A, Sharma R, Prasad R, Bahl A, Khullar M. Role of cardiac TBX20 in dilated cardiomyopathy. *Mol Cell Biochem* 414: 129–136, 2016. doi:10.1007/s11010-016-2666-5.
737. Ahn DG, Ruvinsky I, Oates AC, Silver LM, Ho RK. tbx20, a new vertebrate T-box gene expressed in the cranial motor neurons and developing cardiovascular structures in zebrafish. *Mech Dev* 95: 253–258, 2000. doi:10.1016/S0925-4773(00)00346-4.
738. Stennard FA, Costa MW, Elliott DA, Rankin S, Haast SJ, Lai D, McDonald LP, Niederreither K, Dolle P, Bruneau BG, Zorn AM, Harvey RP. Cardiac T-box factor Tbx20 directly interacts with Nkx2-5, GATA4, and GATA5 in regulation of gene expression in the developing heart. *Dev Biol* 262: 206–224, 2003. doi:10.1016/S0012-1606(03)00385-3.
739. Takeuchi JK, Mileikovskaia M, Koshiba-Takeuchi K, Heidt AB, Mori AD, Arruda EP, Gertsenstein M, Georges R, Davidson L, Mo R, Hui CC, Henkelman RM, Nemer M, Black BL, Nagy A, Bruneau BG. Tbx20 dose-dependently regulates transcription factor networks required for mouse heart and motoneuron development. *Development* 132: 2463–2474, 2005. doi:10.1242/dev.01827.
740. Boogerd CJ, Zhu X, Aneas I, Sakabe N, Zhang L, Sobreira DR, Montefiori L, Bogomolovas J, Joslin AC, Zhou B, Chen J, Nobrega MA, Evans SM. Tbx20 is required in mid-gestation cardiomyocytes and plays a central role in atrial development. *Circ Res* 123: 428–442, 2018. doi:10.1161/CIRCRESAHA.118.311339.
741. Brown DD, Martz SN, Binder O, Goetz SC, Price BM, Smith JC, Conlon FL. Tbx5 and Tbx20 act synergistically to control vertebrate heart morphogenesis. *Development* 132: 553–563, 2005. doi:10.1242/dev.01596.
742. Kennedy L, Kaltenbrun E, Greco TM, Temple B, Herring LE, Cristea IM, Conlon FL. Formation of a TBX20-CASZ1 protein complex is protective against dilated cardiomyopathy and critical for cardiac homeostasis. *PLoS Genet* 13: e1007011, 2017. doi:10.1371/journal.pgen.1007011.
743. Xiang FL, Guo M, Yutzey KE. Overexpression of Tbx20 in adult cardiomyocytes promotes proliferation and improves cardiac function after myocardial infarction. *Circulation* 133: 1081–1092, 2016. doi:10.1161/CIRCULATIONAHA.115.019357.
744. Sakabe NJ, Aneas I, Shen T, Shokri L, Park SY, Bulyk ML, Evans SM, Nobrega MA. Dual transcriptional activator and repressor roles of TBX20 regulate adult cardiac structure and function. *Hum Mol Genet* 21: 2194–2204, 2012. doi:10.1093/hmg/dds034.
745. Shen T, Aneas I, Sakabe N, Dirschinger RJ, Wang G, Smemo S, Westlund JM, Cheng H, Dalton N, Gu Y, Boogerd CJ, Cai CL, Peterson K, Chen J, Nobrega MA, Evans SM. Tbx20 regulates a genetic program essential to adult mouse cardiomyocyte function. *J Clin Invest* 121: 4640–4654, 2011. doi:10.1172/JCI59472.
746. Cai CL, Zhou W, Yang L, Bu L, Qyang Y, Zhang X, Li X, Rosenfeld MG, Chen J, Evans S. T-box genes coordinate regional rates of proliferation and regional specification during cardiogenesis. *Development* 132: 2475–2487, 2005. doi:10.1242/dev.01832.
747. Singh MK, Christoffels VM, Dias JM, Trowe MO, Petry M, Schuster-Gossler K, Bürger A, Ericson J, Kispert A. Tbx20 is essential for cardiac chamber differentiation and repression of Tbx2. *Development* 132: 2697–2707, 2005. doi:10.1242/dev.01854.
748. Stennard FA, Costa MW, Lai D, Biben C, Furtado MB, Solloway MJ, McCulley DJ, Leimena C, Preis JI, Dunwoodie SL, Elliott DE, Prall OW, Black BL, Fatkin D, Harvey RP. Murine T-box transcription factor Tbx20 acts as a repressor during heart development, and is essential for adult heart integrity, function and adaptation. *Development* 132: 2451–2462, 2005. doi:10.1242/dev.01799.
749. Cai X, Nomura-Kitabayashi A, Cai W, Yan J, Christoffels VM, Cai CL. Myocardial Tbx20 regulates early atrioventricular canal formation and endocardial epithelial-mesenchymal transition via Bmp2. *Dev Biol* 360: 381–390, 2011. doi:10.1016/j.ydbio.2011.09.023.
750. Cai X, Zhang W, Hu J, Zhang L, Sultana N, Wu B, Cai W, Zhou B, Cai CL. Tbx20 acts upstream of Wnt signaling to regulate endocardial cushion formation and valve remodeling during mouse cardiogenesis. *Development* 140: 3176–3187, 2013. doi:10.1242/dev.092502.
751. Boogerd CJ, Aneas I, Sakabe N, Dirschinger RJ, Cheng QJ, Zhou B, Chen J, Nobrega MA, Evans SM. Probing chromatin landscape reveals roles of endocardial TBX20 in septation. *J Clin Invest* 126: 3023–3035, 2016. doi:10.1172/JCI85350.
752. Zhang W, Chen H, Wang Y, Yong W, Zhu W, Liu Y, Wagner GR, Payne RM, Field LJ, Xin H, Cai CL, Shou W. Tbx20 transcription factor is a downstream mediator for bone morphogenetic protein-10 in regulating cardiac ventricular wall development and function. *J Biol Chem* 286: 36820–36829, 2011. doi:10.1074/jbc.M111.279679.
753. Chakraborty S, Yutzey KE. Tbx20 regulation of cardiac cell proliferation and-lineage specialization during embryonic and fetal development in vivo. *Dev Biol* 363: 234–246, 2012. doi:10.1016/j.ydbio.2011.12.034.
754. Chakraborty S, Sengupta A, Yutzey KE. Tbx20 promotes cardiomyocyte proliferation and persistence of fetal characteristics in adult mouse hearts. *J Mol Cell Cardiol* 62: 203–213, 2013. doi:10.1016/j.yjmcc.2013.05.018.
755. Arndt AK, Schafer S, Drenckhahn JD, Sabeh MK, Plovie ER, Caliebe A, Klopocki E, Musso G, Werdich AA, Kalwa H, Heinig M, Padera RF, Wassilew K, Bluhm J, Harnack C, Martitz J, Barton PJ, Greutmann M, Berger F, Hubner N, Siebert R, Kramer HH, Cook SA, MacRae CA, Klaassen S. Fine mapping of the 1p36 deletion syndrome identifies mutation of PRDM16 as a cause of cardiomyopathy. *Am J Hum Genet* 93: 67–77, 2013. doi:10.1016/j.ajhg.2013.05.015.
756. Delplancq G, Tarris G, Vitobello A, Nambot S, Sorlin A, Philippe C, Carmignac V, Duffourd Y, Denis C, Eicher JC, Chevarin M, Millat G, Khallouk B, Rousseau T, Falcon-Eicher S, Vasiljevic A, Harizay FT, Thauvin-Robinet C, Faivre L, Kuentz P. Cardiomyopathy due to PRDM16 mutation: First description of a fetal presentation, with possible modifier genes. *Am J Med Genet C Semin Med Genet* 184: 129–135, 2020. doi:10.1002/ajmg.c.31766.
757. Klaassen S, Probst S, Oechslin E, Gerull B, Krings G, Schuler P, Greutmann M, Hürlimann D, Yegitbasi M, Pons L, Gramlich M, Drenckhahn JD, Heuser A, Berger F, Jenni R, Thierfelder L. Mutations in sarcomere protein genes in left ventricular noncompaction. *Circulation* 117: 2893–2901, 2008. doi:10.1161/CIRCULATIONAHA.107.746164.
758. Long PA, Evans JM, Olson TM. Diagnostic yield of whole exome sequencing in pediatric dilated cardiomyopathy. *J Cardiovasc Dev Dis* 4: 11, 2017. doi:10.3390/jcdd4030011.
759. Mazarrotto F, Hawley MH, Beltrami M, Beekman L, de Marvao A, McGurk KA, Statton B, Boschi B, Girolami F, Roberts AM, Lodder EM, Allouba M, Romeih S, Aguib Y, Baksi AJ, Pantazis A, Prasad SK, Cerbai E, Yacoub MH, O'Regan DP, Cook SA, Ware JS, Funke

- B, Olivetto I, Bezzina CR, Barton PJ, Walsh R.** Systematic large-scale assessment of the genetic architecture of left ventricular non-compaction reveals diverse etiologies. *Genet Med* 23: 856–864, 2021. doi:10.1038/s41436-020-01049-x.
760. **Probst S, Oechslin E, Schuler P, Greutmann M, Boyé P, Knirsch W, Berger F, Thierfelder L, Jenni R, Klaassen S.** Sarcomere gene mutations in isolated left ventricular noncompaction cardiomyopathy do not predict clinical phenotype. *Circ Cardiovasc Genet* 4: 367–374, 2011. doi:10.1161/CIRCGENETICS.110.959270.
761. **Shimada S, Shimojima K, Okamoto N, Sangu N, Hirasawa K, Matsuo M, et al.** Microarray analysis of 50 patients reveals the critical chromosomal regions responsible for 1p36 deletion syndrome-related complications. *Brain Dev* 37: 515–526, 2015. doi:10.1016/j.braindev.2014.08.002.
762. **Hong KW, Lim JE, Kim JW, Tabara Y, Ueshima H, Miki T, Matsuda F, Cho YS, Kim Y, Oh B.** Identification of three novel genetic variations associated with electrocardiographic traits (QRS duration and PR interval) in East Asians. *Hum Mol Genet* 23: 6659–6667, 2014. doi:10.1093/hmg/ddu374.
763. **Di Tullio F, Schwarz M, Zorgati H, Mzoughi S, Guccione E.** The duality of PRDM proteins: epigenetic and structural perspectives. *FEBS J* (2021). doi:10.1111/febs.15844.
764. **Fog CK, Galli GG, Lund AH.** PRDM proteins: important players in differentiation and disease. *Bioessays* 34: 50–60, 2012. doi:10.1002/bies.201100107.
765. **Hohenauer T, Moore AW.** The Prdm family: expanding roles in stem cells and development. *Development* 139: 2267–2282, 2012. doi:10.1242/dev.070110.
766. **Chi J, Cohen P.** The multifaceted roles of PRDM16: adipose biology and beyond. *Trends Endocrinol Metab* 27: 11–23, 2016. doi:10.1016/j.tem.2015.11.005.
767. **Nishikata I, Sasaki H, Iga M, Tateno Y, Imayoshi S, Asou N, Nakamura T, Morishita K.** A novel EVI1 gene family, MEL1, lacking a PR domain (MEL1S) is expressed mainly in t(1;3)(p36;q21)-positive AML and blocks G-CSF-induced myeloid differentiation. *Blood* 102: 3323–3332, 2003. doi:10.1182/blood-2002-12-3944.
768. **Cibi DM, Bi-Lin KW, Shekeran SG, Sandireddy R, Tee N, Singh A, Wu Y, Srinivasan DK, Kovalik JP, Ghosh S, Seale P, Singh MK.** Prdm16 deficiency leads to age-dependent cardiac hypertrophy, adverse remodeling, mitochondrial dysfunction, and heart failure. *Cell Rep* 33: 108288, 2020. doi:10.1016/j.celrep.2020.108288.
769. **Wu T, Liang Z, Zhang Z, Liu C, Zhang L, Gu Y, Peterson KL, Evans SM, Fu XD, Chen J.** PRDM16 is a compact myocardium-enriched transcription factor required to maintain compact myocardial cardiomyocyte identity in left ventricle. *Circulation* 2021. doi:10.1161/CIRCULATIONAHA.121.056666.
770. **Ohno H, Shinoda K, Ohyama K, Sharp LZ, Kajimura S.** EHMT1 controls brown adipose cell fate and thermogenesis through the PRDM16 complex. *Nature* 504: 163–167, 2013. doi:10.1038/nature12652.
771. **Harms MJ, Lim HW, Ho Y, Shapira SN, Ishibashi J, Rajakumari S, Steger DJ, Lazar MA, Won KJ, Seale P.** PRDM16 binds MED1 and controls chromatin architecture to determine a brown fat transcriptional program. *Genes Dev* 29: 298–307, 2015. doi:10.1101/gad.252734.114.
772. **Iida S, Chen W, Nakadai T, Ohkuma Y, Roeder RG.** PRDM16 enhances nuclear receptor-dependent transcription of the brown fat-specific Ucp1 gene through interactions with Mediator subunit MED1. *Genes Dev* 29: 308–321, 2015. doi:10.1101/gad.252809.114.
773. **Seale P, Kajimura S, Yang W, Chin S, Rohas LM, Uldry M, Tavernier G, Langin D, Spiegelman BM.** Transcriptional control of brown fat determination by PRDM16. *Cell Metab* 6: 38–54, 2007. doi:10.1016/j.cmet.2007.06.001.
774. **Kajimura S, Seale P, Kubota K, Lunsford E, Frangioni JV, Gygi SP, Spiegelman BM.** Initiation of myoblast to brown fat switch by a PRDM16-C/EBP-beta transcriptional complex. *Nature* 460: 1154–1158, 2009. doi:10.1038/nature08262.
775. **Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scimè A, Devarakonda S, Conroe HM, Erdjument-Bromage H, Tempst P, Rudnicki MA, Beier DR, Spiegelman BM.** PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 454: 961–967, 2008. doi:10.1038/nature07182.
776. **Warner DR, Horn KH, Mudd L, Webb CL, Greene RM, Pisano MM.** PRDM16/MEL1: a novel Smad binding protein expressed in murine embryonic orofacial tissue. *Biochim Biophys Acta* 1773: 814–820, 2007. doi:10.1016/j.bbamcr.2007.03.016.
777. **Takahata M, Inoue Y, Tsuda H, Imoto I, Koinuma D, Hayashi M, Ichikura T, Yamori T, Nagasaki K, Yoshida M, Matsuoka M, Morishita K, Yuki K, Hanyu A, Miyazawa K, Inazawa J, Miyazono K, Imamura T.** SKI and MEL1 cooperate to inhibit transforming growth factor-beta signal in gastric cancer cells. *J Biol Chem* 284: 3334–3344, 2009. doi:10.1074/jbc.M808989200.
778. **Zhou B, Wang J, Lee SY, Xiong J, Bhanu N, Guo Q, Ma P, Sun Y, Rao RC, Garcia BA, Hess JL, Dou Y.** PRDM16 suppresses MLL1r leukemia via intrinsic histone methyltransferase activity. *Mol Cell* 62: 222–236, 2016. doi:10.1016/j.molcel.2016.03.010.
779. **Pinheiro I, Margueron R, Shukeir N, Eisold M, Fritzsche C, Richter FM, Mittler G, Genoud C, Goyama S, Kurokawa M, Son J, Reinberg D, Lachner M, Jenuwein T.** Prdm3 and Prdm16 are H3K9me1 methyltransferases required for mammalian heterochromatin integrity. *Cell* 150: 948–960, 2012. doi:10.1016/j.cell.2012.06.048.
780. **Aguilo F, Avagyan S, Labar A, Sevilla A, Lee DF, Kumar P, Lemischka IR, Zhou BY, Snoeck HW.** Prdm16 is a physiologic regulator of hematopoietic stem cells. *Blood* 117: 5057–5066, 2011. doi:10.1182/blood-2010-08-300145.
781. **Bjork BC, Turbe-Doan A, Prysak M, Herron BJ, Beier DR.** Prdm16 is required for normal palatogenesis in mice. *Hum Mol Genet* 19: 774–789, 2010. doi:10.1093/hmg/ddp543.
782. **Chuikov S, Levi BP, Smith ML, Morrison SJ.** Prdm16 promotes stem cell maintenance in multiple tissues, partly by regulating oxidative stress. *Nat Cell Biol* 12: 999–1006, 2010. doi:10.1038/ncb2101.
783. **Nam JM, Lim JE, Ha TW, Oh B, Kang JO.** Cardiac-specific inactivation of Prdm16 effects cardiac conduction abnormalities and cardiomyopathy-associated phenotypes. *Am J Physiol Heart Circ Physiol* 318: H764–H777, 2020. doi:10.1152/ajpheart.00647.2019.
784. **Bildsoe H, Loebel DA, Jones VJ, Hor AC, Braithwaite AW, Chen YT, Behringer RR, Tam PP.** The mesenchymal architecture of the cranial mesoderm of mouse embryos is disrupted by the loss of Twist1 function. *Dev Biol* 374: 295–307, 2013. doi:10.1016/j.ydbio.2012.12.004.
785. **Saga Y, Kitajima S, Miyagawa-Tomita S.** Mesp1 expression is the earliest sign of cardiovascular development. *Trends Cardiovasc Med* 10: 345–352, 2000. doi:10.1016/S1050-1738(01)00069-X.
786. **Saga Y, Miyagawa-Tomita S, Takagi A, Kitajima S, Miyazaki J, Inoue T.** MesP1 is expressed in the heart precursor cells and required for the formation of a single heart tube. *Development* 126: 3437–3447, 1999. doi:10.1242/dev.126.15.3437.
787. **Breckenridge R, Kotecha S, Towers N, Bennett M, Mohun T.** Panmyocardial expression of Cre recombinase throughout mouse development. *Genesis* 45: 135–144, 2007. doi:10.1002/dvg.20275.
788. **Clegg JS.** Properties and metabolism of the aqueous cytoplasm and its boundaries. *Am J Physiol Regul Integr Comp Physiol* 246: R133–R151, 1984. doi:10.1152/ajpregu.1984.246.2.R133.
789. **Luby-Phelps K.** The physical chemistry of cytoplasm and its influence on cell function: an update. *Mol Biol Cell* 24: 2593–2596, 2013. doi:10.1091/mbc.e12-08-0617.
790. **Luby-Phelps K.** Cytoarchitecture and physical properties of cytoplasm: volume, viscosity, diffusion, intracellular surface area. *Int Rev Cytol* 192: 189–221, 2000. doi:10.1016/s0074-7696(08)60527-6.
791. **Srere PA.** Complexes of sequential metabolic enzymes. *Annu Rev Biochem* 56: 89–124, 1987. doi:10.1146/annurev.bi.56.070187.000513.
792. **Groll M, Clausen T.** Molecular shredders: how proteasomes fulfill their role. *Curr Opin Struct Biol* 13: 665–673, 2003. doi:10.1016/j.sbi.2003.10.005.
793. **Nandi D, Tahiliani P, Kumar A, Chandu D.** The ubiquitin-proteasome system. *J Biosci* 31: 137–155, 2006. doi:10.1007/BF02705243.
794. **Verkman AS.** Solute and macromolecule diffusion in cellular aqueous compartments. *Trends Biochem Sci* 27: 27–33, 2002. doi:10.1016/S0968-0004(01)02003-5.
795. **Kamal A, Goldstein LS.** Principles of cargo attachment to cytoplasmic motor proteins. *Curr Opin Cell Biol* 14: 63–68, 2002. doi:10.1016/S0955-0674(01)00295-2.
796. **Pelham HR.** The Croonian Lecture 1999. Intracellular membrane traffic: getting proteins sorted. *Philos Trans R Soc Lond B Biol Sci* 354: 1471–1478, 1999. doi:10.1098/rstb.1999.0491.
797. **Stryer L, Berg J, Tymoczko J, Gatto G.** *Biochemistry*. New York: Palgrave Macmillan, 2019.

798. **Kholodenko BN.** Four-dimensional organization of protein kinase signaling cascades: the roles of diffusion, endocytosis and molecular motors. *J Exp Biol* 206: 2073–2082, 2003. doi:10.1242/jeb.00298.
799. **Pesaresi P, Schneider A, Kleine T, Leister D.** Interorganellar communication. *Curr Opin Plant Biol* 10: 600–606, 2007. doi:10.1016/j.pbi.2007.07.007.
800. **Bootman MD.** Calcium signaling. *Cold Spring Harb Perspect Biol* 4: a011171, 2012. doi:10.1101/cshperspect.a011171.
801. **Lang F.** Mechanisms and significance of cell volume regulation. *J Am Coll Nutr* 26: 613S–623S, 2007. doi:10.1080/07315724.2007.10719667.
802. **Knezevic T, Myers VD, Gordon J, Tilley DG, Sharp TE 3rd, Wang J, Khalili K, Cheung JY, Feldman AM.** BAG3: a new player in the heart failure paradigm. *Heart Fail Rev* 20: 423–434, 2015. doi:10.1007/s10741-015-9487-6.
803. **Myers VD, McClung JM, Wang J, Tahir FG, Gupta MK, Gordon J, Kontos CH, Khalili K, Cheung JY, Feldman AM.** The multifunctional protein BAG3: a novel therapeutic target in cardiovascular disease. *JACC Basic Transl Sci* 3: 122–131, 2018. doi:10.1016/j.jacbs.2017.09.009.
804. **Cao S, Smith LL, Padilla-Lopez SR, Guida BS, Blume E, Shi J, Morton SU, Brownstein CA, Beggs AH, Kruer MC, Agrawal PB.** Homozygous EEF1A2 mutation causes dilated cardiomyopathy, failure to thrive, global developmental delay, epilepsy and early death. *Hum Mol Genet* 26: 3545–3552, 2017. doi:10.1093/hmg/ddx239.
805. **Kaneko M, Rosser T, Raca G.** Dilated cardiomyopathy in a patient with autosomal dominant EEF1A2-related neurodevelopmental disorder. *Eur J Med Genet* 64: 104121, 2021. doi:10.1016/j.ejmg.2020.104121.
806. **Long K, Wang H, Song Z, Yin X, Wang Y.** EEF1A2 mutations in epileptic encephalopathy/intellectual disability: Understanding the potential mechanism of phenotypic variation. *Epilepsy Behav* 105: 106955, 2020. doi:10.1016/j.yebeh.2020.106955.
807. **Toro R, Pérez-Serra A, Campuzano O, Moncayo-Arlandi J, Allegue C, Iglesias A, Mangas A, Brugada R.** Familial dilated cardiomyopathy caused by a novel frameshift in the BAG3 gene. *PLoS One* 11: e0158730, 2016. doi:10.1371/journal.pone.0158730.
808. **Norton N, Li D, Rieder MJ, Siegfried JD, Rampersaud E, Züchner S, Mangos S, Gonzalez-Quintana J, Wang L, McGee S, Reiser J, Martin E, Nickerson DA, Hershberger RE.** Genome-wide studies of copy number variation and exome sequencing identify rare variants in BAG3 as a cause of dilated cardiomyopathy. *Am J Hum Genet* 88: 273–282, 2011. doi:10.1016/j.ajhg.2011.01.016.
809. **Franszczyk M, Bilinska ZT, Sobieszczanska-Matek M, Michalak E, Sleszycka J, Sioma A, Matek LA, Kaczmarek D, Walczak E, Włodarski P, Hutnik Ł, Milanowska B, Dzielinska Z, Religa G, Grzybowski J, Zieliński T, Płoski R.** The BAG3 gene variants in Polish patients with dilated cardiomyopathy: four novel mutations and a genotype-phenotype correlation. *J Transl Med* 12: 192, 2014. doi:10.1186/1479-5876-12-192.
810. **Domínguez F, Cuenca S, Bilińska Z, Toro R, Villard E, Barriales-Villa R, et al.** Dilated cardiomyopathy due to BLC2-associated atahogene 3 (BAG3) mutations. *J Am Coll Cardiol* 72: 2471–2481, 2018. doi:10.1016/j.jacc.2018.08.2181.
811. **Esslinger U, Garnier S, Korniat A, Proust C, Kararigas G, Müller-Nurasyid M, Empaña JP, et al.** Exome-wide association study reveals novel susceptibility genes to sporadic dilated cardiomyopathy. *PLoS One* 12: e0172995, 2017. doi:10.1371/journal.pone.0172995.
812. **Chami N, Tadros R, Lemarbé F, Lo KS, Beaudoin M, Robb L, Labuda D, Tardif JC, Racine N, Talajic M, Lettre G.** Nonsense mutations in BAG3 are associated with early-onset dilated cardiomyopathy in French Canadians. *Can J Cardiol* 30: 1655–1661, 2014. doi:10.1016/j.cjca.2014.09.030.
813. **Arimura T, Ishikawa T, Nunoda S, Kawai S, Kimura A.** Dilated cardiomyopathy-associated BAG3 mutations impair Z-disc assembly and enhance sensitivity to apoptosis in cardiomyocytes. *Hum Mutat* 32: 1481–1491, 2011. doi:10.1002/humu.21603.
814. **Ellinor PT, Sasse-Klaassen S, Probst S, Gerull B, Shin JT, Toepfel A, Heuser A, Michely B, Yoerger DM, Song BS, Pilz B, Krings G, Coplin B, Lange PE, Dec GW, Hennies HC, Thierfelder L, MacRae CA.** A novel locus for dilated cardiomyopathy, diffuse myocardial fibrosis, and sudden death on chromosome 10q25–26. *J Am Coll Cardiol* 48: 106–111, 2006. doi:10.1016/j.jacc.2006.01.079.
815. **Feldman AM, Begay RL, Knezevic T, Myers VD, Slavov DB, Zhu W, Gowan K, Graw SL, Jones KL, Tilley DG, Coleman RC, Walinsky P, Cheung JY, Mestroni L, Khalili K, Taylor MR.** Decreased levels of BAG3 in a family with a rare variant and in idiopathic dilated cardiomyopathy. *J Cell Physiol* 229: 1697–1702, 2014. doi:10.1002/jcp.24615.
816. **Villard E, Perret C, Gary F, Proust C, Dilanian G, Hengstenberg C, et al.** A genome-wide association study identifies two loci associated with heart failure due to dilated cardiomyopathy. *Eur Heart J* 32: 1065–1076, 2011. doi:10.1093/eurheartj/ehr105.
817. **Konersman CG, Bordini BJ, Scharer G, Lawlor MW, Zangwill S, Southern JF, Amos L, Geddes GC, Kliegman R, Collins MP.** BAG3 myofibrillar myopathy presenting with cardiomyopathy. *Neuromuscul Disord* 25: 418–422, 2015. doi:10.1016/j.nmd.2015.01.009.
818. **Odgerel Z, Sarkozy A, Lee HS, McKenna C, Rankin J, Straub V, Lochmüller H, Paola F, D'Amico A, Bertini E, Bushby K, Goldfarb LG.** Inheritance patterns and phenotypic features of myofibrillar myopathy associated with a BAG3 mutation. *Neuromuscul Disord* 20: 438–442, 2010. doi:10.1016/j.nmd.2010.05.004.
819. **Selcen D, Muntoni F, Burton BK, Pegoraro E, Sewry C, Bite AV, Engel AG.** Mutation in BAG3 causes severe dominant childhood muscular dystrophy. *Ann Neurol* 65: 83–89, 2009. doi:10.1002/ana.21553.
820. **de Denus S, Mottet F, Korol S, Feroz Zada Y, Provost S, Mongrain I, Asselin G, Oussaid E, Busseuil D, Lettre G, Rioux J, Racine N, O'Meara E, White M, Rouleau J, Tardif JC, Dubé MP.** A genetic association study of heart failure: more evidence for the role of BAG3 in idiopathic dilated cardiomyopathy. *ESC Heart Fail* 7: 4384–4389, 2020. doi:10.1002/ehf2.12934.
821. **Aragam KG, Chaffin M, Levinson RT, McDermott G, Choi SH, Shoemaker MB, Haas ME, Weng LC, Lindsay ME, Smith JG, Newton-Cheh C, Roden DM, London B, Wells QS, Ellinor PT, Kathiresan S, Lubitz SA; GRADE Investigators.** Phenotypic refinement of heart failure in a national biobank facilitates genetic discovery. *Circulation* 139: 489–501, 2019. doi:10.1161/CIRCULATIONAHA.118.035774.
822. **Shah S, Henry A, Roselli C, Lin H, Sveinbjörnsson G, Fatemifar G, et al.** Genome-wide association and Mendelian randomisation analysis provide insights into the pathogenesis of heart failure. *Nat Commun* 11: 163, 2020. doi:10.1038/s41467-019-13690-5.
823. **Choquet H, Thai KK, Jiang C, Ranatunga DK, Hoffmann TJ, Go AS, Lindsay AC, Ehm MG, Waterworth DM, Risch N, Schaefer C.** Meta-analysis of 26 638 individuals identifies two genetic loci associated with left ventricular ejection fraction. *Circ Genom Precis Med* 13: e002804, 2020. doi:10.1161/CIRCGEN.119.002804.
824. **Aung N, Vargas JD, Yang C, Cabrera CP, Warren HR, Fung K, Tzanis E, Barnes MR, Rotter JI, Taylor KD, Manichaikul AW, Lima JA, Bluemke DA, Piechnik SK, Neubauer S, Munroe PB, Petersen SE.** Genome-wide analysis of left ventricular image-derived phenotypes identifies fourteen loci associated with cardiac morphogenesis and heart failure development. *Circulation* 140: 1318–1330, 2019. doi:10.1161/CIRCULATIONAHA.119.041161.
825. **Gandhi PU, Gaggin HK, Belcher AM, Harisiades JE, Basile A, Falco A, Rosati A, Piscione F, Januzzi JL Jr, Turco MC.** Analysis of BAG3 plasma concentrations in patients with acutely decompensated heart failure. *Clin Chim Acta* 445: 73–78, 2015. doi:10.1016/j.cca.2015.02.048.
826. **De Marco M, Falco A, Basile A, Rosati A, Festa M, d'Avenia M, Pascale M, Dal Piaz F, Bisogni R, Barcaroli D, Coppola G, Piscione F, Gigantino A, Citro R, De Rosa R, Vitulano G, Virtuoso N, Manganello F, Palermo E, Siano F, Rosato G, Hahne M, Tiberti C, De Laurenzi V, Turco MC.** Detection of soluble BAG3 and anti-BAG3 antibodies in patients with chronic heart failure. *Cell Death Dis* 4: e495, 2013. doi:10.1038/cddis.2013.8.
827. **Doong H, Price J, Kim YS, Gasbarre C, Probst J, Liotta LA, Blanchette J, Rizzo K, Kohn E.** CAIR-1/BAG-3 forms an EGF-regulated ternary complex with phospholipase C-gamma and Hsp70/Hsc70. *Oncogene* 19: 4385–4395, 2000. doi:10.1038/sj.onc.1203797.
828. **Doong H, Vrailas A, Kohn EC.** What's in the 'BAG'?—A functional domain analysis of the BAG-family proteins. *Cancer Lett* 188: 25–32, 2002. doi:10.1016/S0304-3835(02)00456-1.
829. **Rauch JN, Gestwicki JE.** Binding of human nucleotide exchange factors to heat shock protein 70 (Hsp70) generates functionally

- distinct complexes in vitro. *J Biol Chem* 289: 1402–1414, 2014. doi:10.1074/jbc.M113.521997.
830. Takayama S, Xie Z, Reed JC. An evolutionarily conserved family of Hsp70/Hsc70 molecular chaperone regulators. *J Biol Chem* 274: 781–786, 1999. doi:10.1074/jbc.274.2.781.
831. Lee JH, Takahashi T, Yasuhara N, Inazawa J, Kamada S, Tsujimoto Y. Bis, a Bcl-2-binding protein that synergizes with Bcl-2 in preventing cell death. *Oncogene* 18: 6183–6190, 1999. doi:10.1038/sj.onc.1203043.
832. Takayama S, Sato T, Krajewski S, Kochel K, Irie S, Millan JA, Reed JC. Cloning and functional analysis of BAG-1: a novel Bcl-2-binding protein with anti-cell death activity. *Cell* 80: 279–284, 1995. doi:10.1016/0092-8674(95)90410-7.
833. Colvin TA, Gabai VL, Gong J, Calderwood SK, Li H, Gummuluru S, Matchuk ON, Smirnova SG, Orlova NV, Zamulaeva IA, Garcia-Marcos M, Li X, Young ZT, Rauch JN, Gestwicki JE, Takayama S, Sherman MY. Hsp70-Bag3 interactions regulate cancer-related signaling networks. *Cancer Res* 74: 4731–4740, 2014. doi:10.1158/0008-5472.CAN-14-0747.
834. Carra S, Seguin SJ, Lambert H, Landry J. HspB8 chaperone activity toward poly(Q)-containing proteins depends on its association with Bag3, a stimulator of macroautophagy. *J Biol Chem* 283: 1437–1444, 2008. doi:10.1074/jbc.M706304200.
835. Fang X, Bogomolovas J, Trexler C, Chen J. The BAG3-dependent and -independent roles of cardiac small heat shock proteins. *JCI Insight* 4: e126464, 2019. doi:10.1172/jci.insight.126464.
836. Fuchs M, Poirier DJ, Seguin SJ, Lambert H, Carra S, Charette SJ, Landry J. Identification of the key structural motifs involved in HspB8/HspB6-Bag3 interaction. *Biochem J* 425: 245–255, 2009. [Erratum in *Biochem J* 430: 559, 2010]. doi:10.1042/BJ20090907.
837. Hishiya A, Salman MN, Carra S, Kampinga HH, Takayama S. BAG3 directly interacts with mutated alphaB-crystallin to suppress its aggregation and toxicity. *PLoS One* 6: e16828, 2011. doi:10.1371/journal.pone.0016828.
838. Rauch JN, Tse E, Freilich R, Mok SA, Makley LN, Southworth DR, Gestwicki JE. BAG3 is a modular, scaffolding protein that physically links heat shock protein 70 (Hsp70) to the small heat shock proteins. *J Mol Biol* 429: 128–141, 2017. doi:10.1016/j.jmb.2016.11.013.
839. Liu L, Sun K, Zhang X, Tang Y, Xu D. Advances in the role and mechanism of BAG3 in dilated cardiomyopathy. *Heart Fail Rev* 26: 183–194, 2021. doi:10.1007/s10741-019-09899-7.
840. Fang X, Bogomolovas J, Wu T, Zhang W, Liu C, Veevers J, Stroud MJ, Zhang Z, Ma X, Mu Y, Lao DH, Dalton ND, Gu Y, Wang C, Wang M, Liang Y, Lange S, Ouyang K, Peterson KL, Evans SM, Chen J. Loss-of-function mutations in co-chaperone BAG3 destabilize small HSPs and cause cardiomyopathy. *J Clin Invest* 127: 3189–3200, 2017. doi:10.1172/JCI94310.
841. Hishiya A, Kitazawa T, Takayama S. BAG3 and Hsc70 interact with actin capping protein CapZ to maintain myofibrillar integrity under mechanical stress. *Circ Res* 107: 1220–1231, 2010. doi:10.1161/CIRCRESAHA.110.225649.
842. Feldman AM, Gordon J, Wang J, Song J, Zhang XQ, Myers VD, Tilley DG, Gao E, Hoffman NE, Tomar D, Madesh M, Rabinowitz J, Koch WJ, Su F, Khalili K, Cheung JY. BAG3 regulates contractility and Ca²⁺ homeostasis in adult mouse ventricular myocytes. *J Mol Cell Cardiol* 92: 10–20, 2016. doi:10.1016/j.yjmcc.2016.01.015.
843. Martin TG, Myers VD, Dubey P, Dubey S, Perez E, Moravec CS, Willis MS, Feldman AM, Kirk JA. Cardiomyocyte contractile impairment in heart failure results from reduced BAG3-mediated sarcomeric protein turnover. *Nat Commun* 12: 2942, 2021. doi:10.1038/s41467-021-23272-z.
844. Homma S, Iwasaki M, Shelton GD, Engvall E, Reed JC, Takayama S. BAG3 deficiency results in fulminant myopathy and early lethality. *Am J Pathol* 169: 761–773, 2006. doi:10.2353/ajpath.2006.060250.
845. Youn DY, Lee DH, Lim MH, Yoon JS, Lim JH, Jung SE, Yeum CE, Park CW, Youn HJ, Lee JS, Lee SB, Ikawa M, Okabe M, Tsujimoto Y, Lee JH. Bis deficiency results in early lethality with metabolic deterioration and involution of spleen and thymus. *Am J Physiol Endocrinol Metab* 295: E1349–E1357, 2008. doi:10.1152/ajpendo.90704.2008.
846. Minoia M, Grit C, Kampinga HH. HSPA1A-independent suppression of PARK2 C289G protein aggregation by human small heat shock proteins. *Mol Cell Biol* 34: 3570–3578, 2014. doi:10.1128/MCB.00698-14.
847. Vos MJ, Zijlstra MP, Kanon B, van Waarde-Verhagen MA, Brunt ER, Oosterveld-Hut HM, Carra S, Sibon OC, Kampinga HH. HSPB7 is the most potent polyQ aggregation suppressor within the HSPB family of molecular chaperones. *Hum Mol Genet* 19: 4677–4693, 2010. doi:10.1093/hmg/ddq398.
848. Wu T, Mu Y, Bogomolovas J, Fang X, Veevers J, Nowak RB, Pappas CT, Gregorio CC, Evans SM, Fowler VM, Chen J. HSPB7 is indispensable for heart development by modulating actin filament assembly. *Proc Natl Acad Sci USA* 114: 11956–11961, 2017. doi:10.1073/pnas.1713763114.
849. Myers VD, Tomar D, Madesh M, Wang J, Song J, Zhang XQ, Gupta MK, Tahir FG, Gordon J, McClung JM, Kontos CD, Khalili K, Cheung JY, Feldman AM. Haplo-insufficiency of Bcl2-associated athanogene 3 in mice results in progressive left ventricular dysfunction, beta-adrenergic insensitivity, and increased apoptosis. *J Cell Physiol* 233: 6319–6326, 2018. doi:10.1002/jcp.26482.
850. Jia P, Wu N, Yang H, Guo Y, Guo X, Sun Y. Different roles of BAG3 in cardiac physiological hypertrophy and pathological remodeling. *Transl Res* 233: 47–61, 2021. doi:10.1016/j.trsl.2021.02.004.
851. Inomata Y, Nagasaka S, Miyate K, Goto Y, Hino C, Toukairin C, Higashio R, Ishida K, Saino T, Hirose M, Tsumura H, Sanbe A. Bcl-2-associated athanogene 3 (BAG3) is an enhancer of small heat shock protein turnover via activation of autophagy in the heart. *Biochem Biophys Res Commun* 496: 1141–1147, 2018. doi:10.1016/j.bbrc.2018.01.158.
852. Fang X, Bogomolovas J, Zhou PS, Mu Y, Ma X, Chen Z, Zhang L, Zhu M, Veevers J, Ouyang K, Chen J. P209L mutation in Bag3 does not cause cardiomyopathy in mice. *Am J Physiol Heart Circ Physiol* 316: H392–H399, 2019. doi:10.1152/ajpheart.00714.2018.
853. Quintana MT, Parry TL, He J, Yates CC, Sidorova TN, Murray KT, Bain JR, Newgard CB, Muehlbauer MJ, Eaton SC, Hishiya A, Takayama S, Willis MS. Cardiomyocyte-specific human Bcl2-associated athanogene 3 P209L expression induces mitochondrial fragmentation, Bcl2-associated athanogene 3 haploinsufficiency, and activates p38 signaling. *Am J Pathol* 186: 1989–2007, 2016. doi:10.1016/j.ajpath.2016.03.017.
854. Kimura K, Ooms A, Graf-Riesen K, Kuppusamy M, Unger A, Schuld J, Daerr J, Lothar A, Geisen C, Hein L, Takahashi S, Li G, Röhl W, Bloch W, van der Ven PF, Linke WA, Wu SM, Huesgen PF, Höhfeld J, Fürst DO, Fleischmann BK, Hesse M. Overexpression of human BAG3(P209L) in mice causes restrictive cardiomyopathy. *Nat Commun* 12: 3575, 2021. doi:10.1038/s41467-021-23858-7.
855. Voorhees RM, Ramakrishnan V. Structural basis of the translational elongation cycle. *Annu Rev Biochem* 82: 203–236, 2013. doi:10.1146/annurev-biochem-113009-092313.
856. Khalyfa A, Bourbeau D, Chen E, Petroulakis E, Pan J, Xu S, Wang E. Characterization of elongation factor-1A (eEF1A-1) and eEF1A-2/S1 protein expression in normal and wasted mice. *J Biol Chem* 276: 22915–22922, 2001. doi:10.1074/jbc.M101011200.
857. Lee S, Francoeur AM, Liu S, Wang E. Tissue-specific expression in mammalian brain, heart, and muscle of S1, a member of the elongation factor-1 alpha gene family. *J Biol Chem* 267: 24064–24068, 1992. doi:10.1016/S0021-9258(18)35946-5.
858. Newbery HJ, Loh DH, O'Donoghue JE, Tomlinson VAL, Chau YY, Boyd JA, Bergmann JH, Brownstein D, Abbott CM. Translation elongation factor eEF1A2 is essential for post-weaning survival in mice. *J Biol Chem* 282: 28951–28959, 2007. doi:10.1074/jbc.M703962200.
859. Soares DC, Barlow PN, Newbery HJ, Porteous DJ, Abbott CM. Structural models of human eEF1A1 and eEF1A2 reveal two distinct surface clusters of sequence variation and potential differences in phosphorylation. *PLoS One* 4: e6315, 2009. doi:10.1371/journal.pone.0006315.
860. Carriles AA, Mills A, Muñoz-Alonso MJ, Gutiérrez D, Domínguez JM, Hermoso JA, Gago F. Structural cues for understanding eEF1A2 moonlighting. *Chembiochem* 22: 374–391, 2021. doi:10.1002/cbic.202000516.
861. Pinke DE, Lee JM. The lipid kinase PI4KIIIbeta and the eEF1A2 oncogene co-operate to disrupt three-dimensional in vitro acinar morphogenesis. *Exp Cell Res* 317: 2503–2511, 2011. doi:10.1016/j.yexcr.2011.08.002.
862. Jeganathan S, Morrow A, Amiri A, Lee JM. Eukaryotic elongation factor 1A2 cooperates with phosphatidylinositol-4 kinase III beta to stimulate production of filopodia through increased phosphatidylinositol-4,5

- bisphosphate generation. *Mol Cell Biol* 28: 4549–4561, 2008. doi:10.1128/MCB.00150-08.
863. **Panasuk G, Nemazany I, Filonenko V, Negrutskii B, El'skaya AV.** A2 isoform of mammalian translation factor eEF1A displays increased tyrosine phosphorylation and ability to interact with different signalling molecules. *Int J Biochem Cell Biol* 40: 63–71, 2008. doi:10.1016/j.biocel.2007.08.014.
864. **Chambers DM, Peters J, Abbott CM.** The lethal mutation of the mouse wasted (*wst*) is a deletion that abolishes expression of a tissue-specific isoform of translation elongation factor 1 α , encoded by the *Eef1a2* gene. *Proc Natl Acad Sci USA* 95: 4463–4468, 1998. doi:10.1073/pnas.95.8.4463.
865. **Doig J, Griffiths LA, Peberdy D, Dharmasaroja P, Vera M, Davies FJ, Newbery HJ, Brownstein D, Abbott CM.** In vivo characterization of the role of tissue-specific translation elongation factor 1A2 in protein synthesis reveals insights into muscle atrophy. *FEBS J* 280: 6528–6540, 2013. doi:10.1111/febs.12554.
866. **Feng W, Wang L, Veevers J, Liu C, Huang T, Chen J.** Loss of eEF1A2 (Eukaryotic Elongation Factor 1 A2) in murine myocardium results in dilated cardiomyopathy. *Circ Heart Fail* 14: e008665, 2021. doi:10.1161/CIRCHEARTFAILURE.121.008665.
867. **Ma X, Chen C, Veevers J, Zhou X, Ross RS, Feng W, Chen J.** CRISPR/Cas9-mediated gene manipulation to create single-amino-acid-substituted and floxed mice with a cloning-free method. *Sci Rep* 7: 42244, 2017. doi:10.1038/srep42244.
868. **Kostera-Pruszczyk A, Suszek M, Płoski R, Franaszczuk M, Potulska-Chromik A, Pruszczyk P, Sadurska E, Karolczak J, Kamińska AM, Rełodowicz MJ.** BAG3-related myopathy, polyneuropathy and cardiomyopathy with long QT syndrome. *J Muscle Res Cell Motil* 36: 423–432, 2015. doi:10.1007/s10974-015-9431-3.
869. **Fang X, Bogomolovas J, Wu T, Zhang W, Liu C, Veevers J, Stroud MJ, Zhang Z, Ma X, Mu Y, Lao DH, Dalton ND, Gu Y, Wang C, Wang M, Liang Y, Lange S, Ouyang K, Peterson KL, Evans SM, Chen J.** Loss-of-function mutations in co-chaperone BAG3 destabilize small HSPs and cause cardiomyopathy. *J Clin Invest* 127: 3189–3200, 2017. doi:10.1172/JCI94310.
870. **Montagutelli X.** Effect of the genetic background on the phenotype of mouse mutations. *J Am Soc Nephrol* 11: S101–S105, 2000. doi:10.1681/ASN.V11suppl_2s101.
871. **Doetschman T.** Influence of genetic background on genetically engineered mouse phenotypes. *Methods Mol Biol* 530: 423–433, 2009. doi:10.1007/978-1-59745-471-1_23.
872. **Taylor MR, Adler ED.** Danon disease. In: *GeneReviews*, edited by Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJ, Mirzaa G, Amemiya A. Seattle WA: Univ. of Washington, 1993.
873. **Cenacchi G, Papa V, Pegoraro V, Marozzo R, Fanin M, Angelini C.** Review: Danon disease: Review of natural history and recent advances. *Neuropathol Appl Neurobiol* 46: 303–322, 2020. doi:10.1111/nan.12587.
874. **Manso AM, Hashem SI, Nelson BC, Gault E, Soto-Hermida A, Villarruel E, Brambatti M, Bogomolovas J, Bushway PJ, Chen C, Battiprolu P, Keravala A, Schwartz JD, Shah G, Gu Y, Dalton ND, Hammond K, Peterson K, Saftig P, Adler ED.** Systemic AAV9. LAMP2B injection reverses metabolic and physiologic multiorgan dysfunction in a murine model of Danon disease. *Sci Transl Med* 12: eaax1744, 2020. doi:10.1126/scitranslmed.aax1744.
875. **Tanaka Y, Guhde G, Suter A, Eskelinen EL, Hartmann D, Lüllmann-Rauch R, Janssen PM, Blanz J, von Figura K, Saftig P.** Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficient mice. *Nature* 406: 902–906, 2000. doi:10.1038/35022595.
876. **Knollmann BC, Chopra N, Hlaing T, Akin B, Yang T, Etensohn K, Knollmann BE, Horton KD, Weissman NJ, Holinstat I, Zhang W, Roden DM, Jones LR, Franzini-Armstrong C, Pfeifer K.** Casq2 deletion causes sarcoplasmic reticulum volume increase, premature Ca²⁺ release, and catecholaminergic polymorphic ventricular tachycardia. *J Clin Invest* 116: 2510–2520, 2006. doi:10.1172/JCI29128.
877. **Watanabe H, Chopra N, Laver D, Hwang HS, Davies SS, Roach DE, Duff HJ, Roden DM, Wilde AA, Knollmann BC.** Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in mice and humans. *Nat Med* 15: 380–383, 2009. doi:10.1038/nm.1942.
878. **Milani-Nejad N, Janssen PM.** Small and large animal models in cardiac contraction research: advantages and disadvantages. *Pharmacol Ther* 141: 235–249, 2014. doi:10.1016/j.pharmthera.2013.10.007.
879. **Riehle C, Bauersachs J.** Key inflammatory mechanisms underlying heart failure. *Herz* 44: 96–106, 2019. doi:10.1007/s00059-019-4785-8.
880. **Rau CD, Lusic AJ, Wang Y.** Systems genetics for mechanistic discovery in heart diseases. *Circ Res* 126: 1795–1815, 2020. doi:10.1161/CIRCRESAHA.119.315863.
881. **Coley WD, Bogdanik L, Vila MC, Yu Q, Van Der Meulen JH, Rayavarapu S, Novak JS, Nearing M, Quinn JL, Saunders A, Dolan C, Andrews W, Lammert C, Austin A, Partridge TA, Cox GA, Lutz C, Nagaraju K.** Effect of genetic background on the dystrophic phenotype in mdx mice. *Hum Mol Genet* 25: 130–145, 2016. doi:10.1093/hmg/ddv460.
882. **Ding Y, Bu H, Xu X.** Modeling inherited cardiomyopathies in adult zebrafish for precision medicine. *Front Physiol* 11: 599244, 2020. doi:10.3389/fphys.2020.599244.
883. **Rieblinger B, Sid H, Duda D, Bozoglu T, Klinger R, Schlickerrieder A, Lengyel K, Flisikowski K, Flisikowska T, Simm N, Grodzicki A, Perleberg C, Bähr A, Carrier L, Kurome M, Zakhartchenko V, Kessler B, Wolf E, Kettler L, Luksch H, Hagag IT, Wise D, Kaufman J, Kaufner BB, Kupatt C, Schnieke A, Schusser B.** Cas9-expressing chickens and pigs as resources for genome editing in livestock. *Proc Natl Acad Sci USA* 118: e2022562118, 2021. doi:10.1073/pnas.2022562118.
884. **Almomani R, Verhagen JM, Herkert JC, Brosens E, van Spaendonck-Zwarts KY, Asimaki A, van der Zwaag PA, Frohn-Mulder IM, Bertoli-Avella AM, Boven LG, van Slegtenhorst MA, van der Smagt JJ, van IJcken WF, Timmer B, van Stuijvenberg M, Verdijk RM, Saffitz JE, du Plessis FA, Michels M, Hofstra RM, Sinke RJ, van Tintelen JP, Wessels MW, Jongbloed JD, van de Laar IM.** Biallelic truncating mutations in ALPK3 cause severe pediatric cardiomyopathy. *J Am Coll Cardiol* 67: 515–525, 2016. doi:10.1016/j.jacc.2015.10.093.
885. **Cheawsamoot C, Phokaew C, Chetruengchai W, Chantranuwat P, Puwanant S, Tongsimma S, Khongphatthanayothin A, Shotelersuk V.** A pathogenic variant in ALPK3 is associated with an autosomal dominant adult-onset hypertrophic cardiomyopathy. *Circ Genom Precis Med* 13: e003127, 2020. doi:10.1161/CIRCGEN.120.003127.
886. **Herkert JC, Verhagen JM, Yotti R, Haghghi A, Phelan DG, James PA, et al.** Expanding the clinical and genetic spectrum of ALPK3 variants: phenotypes identified in pediatric cardiomyopathy patients and adults with heterozygous variants. *Am Heart J* 225: 108–119, 2020. doi:10.1016/j.ahj.2020.03.023.
887. **Jorholt J, Formicheva Y, Vershinina T, Kiselev A, Muravyev A, Demchenko E, Fedotov P, Zlotina A, Rygkov A, Vasichkina E, Sejersen T, Kostareva A.** Two new cases of hypertrophic cardiomyopathy and skeletal muscle features associated with ALPK3 homozygous and compound heterozygous variants. *Genes (Basel)* 11: 1201, 2020. doi:10.3390/genes11101201.
888. **Lopes LR, Garcia-Hernández S, Lorenzini M, Futema M, Chumakova O, Zateyshchikov D, et al.** Alpha-protein kinase 3 (ALPK3) truncating variants are a cause of autosomal dominant hypertrophic cardiomyopathy. *Eur Heart J* 42: 3063–3073, 2021. doi:10.1093/eurheartj/ehab424.
889. **Reinstein E, Orvin K, Tayeb-Fligelman E, Stiebel-Kalish H, Tzur S, Pimienta AL, Bazak L, Bengal T, Cohen L, Gatton DD, Bormans C, Landau M, Kornowski R, Shohat M, Behar DM.** Mutations in TAX1BP3 cause dilated cardiomyopathy with septo-optic dysplasia. *Hum Mutat* 36: 439–442, 2015. doi:10.1002/humu.22759.
890. **Cerrone M, Remme CA, Tadros R, Bezzina CR, Delmar M.** Beyond the one gene-one disease paradigm: complex genetics and pleiotropy in inheritable cardiac disorders. *Circulation* 140: 595–610, 2019. doi:10.1161/CIRCULATIONAHA.118.035954.
891. **Gacita AM, Fullenkamp DE, Ohiri J, Pottinger T, Puckelwartz MJ, Nobrega MA, McNally EM.** Genetic variation in enhancers modifies cardiomyopathy gene expression and progression. *Circulation* 143: 1302–1316, 2021. doi:10.1161/CIRCULATIONAHA.120.050432.
892. **Gifford CA, Ranade SS, Samarakoon R, Salunga HT, de Soysa TY, Huang Y, Zhou P, Eifenbein A, Wyman SK, Bui YK, Cordes Metzler KR, Ursell P, Ivey KN, Srivastava D.** Oligogenic inheritance of a human heart disease involving a genetic modifier. *Science* 364: 865–870, 2019. doi:10.1126/science.aat5056.
893. **Swaggart KA, McNally EM.** Modifiers of heart and muscle function: where genetics meets physiology. *Exp Physiol* 99: 621–626, 2014. doi:10.1113/expphysiol.2013.075887.
894. **Salman OF, El-Rayess HM, Abi Khalil C, Nemer G, Refaat MM.** Inherited cardiomyopathies and the role of mutations in non-coding regions of the genome. *Front Cardiovasc Med* 5: 77, 2018. doi:10.3389/fcvm.2018.00077.