Vinculin and Talin: Focus on the Myocardium

Alice Zemljic-Harpf, MD,† Ana Maria Manso, PhD,*† and Robert S. Ross, MD*†*

Abstract: Cardiomyopathy is a heart muscle disease caused by decreased contractility of the ventricles leading to heart failure and premature death. Multiple conditions like ischemic heart disease (atherosclerosis), hypertension, diabetes, viral infection, alcohol abuse, obesity and genetic mutations can lead to cardiomyopathy. Single gene mutations in sarcomeric proteins, Z-disk-associated proteins, membrane/associated proteins, intermediate filaments, calcium cycle proteins as well as in modifier genes have been linked to cardiomyopathy. Clinical practice guidelines have been formulated by the American Heart Association and the Heart Failure Association of America on how to genetically evaluate patients with cardiomyopathy. To illustrate the concept that alterations in genes cause cardiovascular disease, this review will focus on two membrane-associated proteins, vinculin and talin. We will discuss the general function of vinculin/metavinulin as well as talin1 and talin2, with emphasis on what is understood about their role in the cardiac myocyte and in whole heart.

Key Words: vinculin, talin, costamere, intercalated disk, mechanotransduction

(*J Investig Med* 2009;57: 849–855)

Evidence linking genetic causes to heart muscle diseases has only arisen in the last several decades, with hypertrophic cardiomyopathies being the subtype most extensively linked to single gene defects.[1–4] In more recent time, all major forms of cardiomyopathies have been shown to have a genetic basis, leading to enough scientific traction that clinical practice guidelines have been formulated by the American Heart Association and the Heart Failure Association of America on genetic evaluation of cardiomyopathies.[5,6] Mutations in genes from various classes have been identified as causal of these diseases including one encoding sarcomeric proteins, those important for muscle metabolism or that can lead to infiltrative diseases of the heart, ones critical for proper excitation-contraction coupling of the myocyte or that are present in myocyte cytoskeleton, and also proteins localized to the cardiac myocyte costamere. Our laboratory has sought to elucidate the basic role of costameric proteins in cardiomyocyte function and, in this review, we will focus on two of these proteins, vinculin (Vcl) and talin (Tln).

What is a Costamere?

Costameres are subsarcolemmal structures in striated muscle that circumferentially align with the Z disk of the myofibrils. They function to allow muscle adhesion to the extracellular matrix (ECM). These structures resemble focal adhesions (FAs) found in nonmuscle cells, sharing with them many protein components such as integrins, α-actinin, FA kinase (FAK), integrin-linked kinase, parvin, the particularly interesting cysteine- and histidine-rich protein, Vcl, and Tln.[7]

Besides their structural role in cell attachment, costameres have been shown to be sites where contractile forces generated by cardiomyocytes are transmitted to the ECM (inside out)[8] and where forces externally applied by the ECM are transmitted into the myocyte (outside in).[9] Therefore, costameres are sites where mechanical information is transduced bidirectionally across the cell membrane. Transmission using the outside-in mechanism may allow for the hypertrophic response of myocytes subjected to hemodynamic load. Still, it must be appreciated that myocyte mechanotransduction is not restricted to the costamere but may also occur at Z disks and intercalated disks (ICDs) within the cell.[10] Within the costamere are 2 main protein complexes: the integrin complex and the dystrophin-glycoprotein complex[11] (DGC; Fig. 1). Defects or mutations in proteins of both structures lead to cardiomyopathies, indicating the importance of costameres in normal cardiac function and myocardial remodeling.[12]

The DGC is composed of several membrane-spanning and associated proteins. In the muscle, the DGC includes dystrophin, sarcoglycans (α, β, γ, δ, ε, and ζ), dystroglycans (α and β), α-dystrobrevin, syntrophins (α1, β1, and β2), sarcospan, and nitric oxide synthase.[11] Dystrophin is a 427-kd protein that constitutes a core component of the DGC. The DGC functions to anchor the sarcolemma to the ECM and the sarcomere. It stabilizes the sarcolemma against physical forces during muscle contraction or stretch. Mutations in the many components of the DGC in man and in animal models have been shown to cause a variety of forms of skeletal muscular dystrophy and dilated cardiomyopathy.[11,13,14] A comprehensive discussion of the DGC proteins is outside the scope of this paper, and the reader is referred to some excellent recent reviews.[11,15]

The other main complex in costameres is the integrin complex (Fig. 1). Integrins are heterodimeric surface receptors composed of α and β subunits that bridge the cytoskeleton and, perhaps, the muscle sarcomere with the ECM. They provide for cellular adhesion and also act as mechanotransducers, converting mechanical stimuli to biochemical ones.[16] Adult heart cardiac myocytes express α7β1 (a laminin receptor) as the main integrin, whereas α5β1 and α6β1 are expressed highly in myocytes during heart development. The function of integrins in cardiac myocytes has been analyzed by our own group and others, using both in vitro and in vivo studies. β1 has been linked to a hypertrophic phenotype using a neonatal rat cardiac myocytes model system.[17] Transgenic and knockout (KO) mouse models have also shown how integrin function within the myocyte is essential for preservation of normal heart function.[18–21] These data indicate that the connection of the ECM with the cytoskeleton/sarcomere provided by myocyte integrins is essential for accommodation to the transmission of the mechanical stress that occurs continuously in cardiac myocytes.

The connection of integrins with the actin filaments is indirect and occurs through several structural proteins such as Tln, Vcl, α-actinin, ILK, filamin, and tensin.[22] Besides these structural connections, integrins also recruit signaling proteins

---

From the *Department of Medicine, UCSD School of Medicine, La Jolla; and †Veterans Administration San Diego Healthcare System, San Diego, CA.

Received September 9, 2009, and in revised form October 8, 2009.
Accepted for publication October 8, 2009.

Reprints: Robert S. Ross, MD, Cardiology Section, Veterans Administration San Diego Healthcare System, 111A, 3350 La Jolla Village Dr, San Diego, CA 92161. E-mail: rross@ucsd.edu.

This work was supported by grants to R.S.R. from the Veterans Administration (VA Merit) and the National Institutes of Health (P01 HL066941 and RO1 HL088390), and the symposium was supported in part by a grant from the National Center for Research Resources (R13 RR023236).

ISSN: 1081-5589
DOI: 10.231/JIM.0b013e3181c5e074

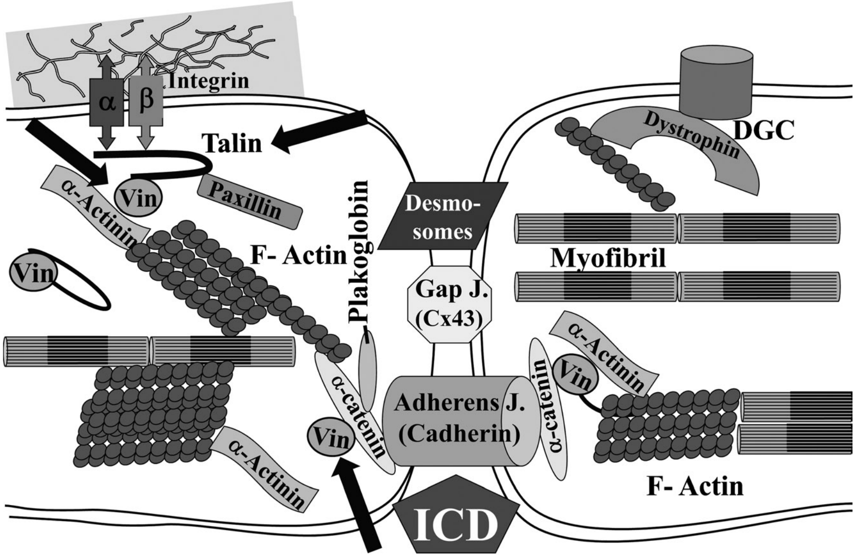

FIGURE 1. Costameric proteins and the ICD of 2 adjacent cardiac myocytes. Shown are some of the key structural elements of the costameric protein complex of the cardiac myocyte, including ones linked to the integrin-Tln-Vcl axis and the dystrophic-glycoprotein complex (DGC). The ICD structure providing mechanical and electrical linkage of myocytes is also shown. The ICD mechanical linkages include the desmosomes and the adherens (cadherin-containing) junctions, whereas electrical coupling of cells occurs at gap junctions (Gap J.) that contain Cxs such as Cx43. This diagram is provided to orient the reader to the location of Vcl and Tln (arrows) within the cell but is clearly a simplified version of costamere and ICD.

that function in the transduction of mechanical stimuli. Important integrin-mediated signaling events occur through FAK, melusin, paxillin, src, cas, and phosphatidylinositol 4-phosphate 5-kinase type I gamma.[23] For a discussion of integrin function and signaling, particularly as it relates to the heart, the reader is referred to recent publications.[24–26] This review will focus on 2 proteins that directly or indirectly connect to integrins: Vcl and Tln.

Vinculin: General Structure and Function

Vinculin is a ubiquitously expressed, membrane-associated protein that links the actin cytoskeleton to the sarcolemma. A splice-variant isoform of Vcl, termed metavinculin (MVcl), exists in the muscle and platelets. Metavinculin arises from a 68–amino acid insertion at residue 915 in the 1066 Vcl sequence, thus producing this 1134 AA form.[27] Some work indicates that Vcl and MVcl colocalize in cardiac myocytes.[28] Their identical head regions interact with membrane-associated proteins, but the unique tail regions of each isoform provide for varied actin organization.[28]

Because Vcl directly binds to the integrin-associated protein Tln and α-actinin as well as to the cadherin-associated proteins α/β-catenins, it is found in cell-matrix and cell-cell contact sites.[29–33] In cardiac myocytes, Vcl protein expression was first described in a transverse, riblike pattern. Because the Latin term for rib is *costa*, the location of this cell-matrix adhesion site of Vcl came to be defined as the *costamere*.[34] Vinculin binds indirectly to integrins through Tln (see later); thus, it functions as a multiprotein linker connecting cell-matrix adhesions to the actin-based cytoskeleton.[35,36] Cell-cell junctions in cardiac myocytes are called ICDs (Fig. 1). The ICD connects myocytes end-to-end in a staggered fashion and contains structures essential for both mechanical and electrical coupling. These include actin-binding adherens junctions, intermediate filament interacting desmosomes, and action potential–conducting gap junctions.[37] Vinculin is highly expressed in ICDs and the costamere. Costameres and ICDs anchor contractile filaments to the sarcolemma and can be thought of as not only cellular junctions important for force transmission but also as potential areas of force generation.

Vinculin's crystal structure has revealed a globular head followed by a hinge region and a flexible tail.[38–40] Intramolecular head-to-tail association masks binding sites within Vcl, thereby keeping the protein in an inactive state.[41] Molecules such as acidic phospholipids,[42,43] Tln,[44] and α-actinin[45] were found to unmask Vcl's binding sites, allowing for Vcl activation.[46] Once activated, Vcl interacts with multiple proteins including Tln, paxillin, α/β-catenin, Arp 2/3, vasodilator-stimulated phosphoprotein (VASP), vinexin, alpha-actinin, F-actin, alpha-synemin, protein kinase Cα (PKCα), acidic phospholipids, and Raver1.[46] The interaction of Vcl and Raver1 is unique, because Raver1 harbors RNA recognition motifs and regulates messenger RNA processing.[47,48]

Studies of Vcl-deficient embryonic carcinoma cells found that they had poor cell-substrate adhesion and absent lamellipodia formation,[49,50] suggesting that Vcl may stabilize matrix-adhesion sites by transferring mechanical stress, which drive cytoskeletal remodeling.[51] Although *Vcl*-null embryonic fibroblasts still formed FAs, these adhesions were smaller, less stable, and turned over more rapidly than those in wild-type cells.[52] Contractile arrest of neonatal cardiac myocytes led to depletion of Vcl from the costameres, and mechanical stress increased Vcl expression at these FA sites.[53] Taken together, these data indicate that Vcl (and by extension MVcl) mechanically couples the actin-based cytoskeleton to the sarcolemma and regulates FA turnover.

To extend these cellular studies to the whole organism, the *Vcl* gene was globally ablated in the mouse genome.[54] With this, growth retardation of the embryo was evident from embryonic day (E)8 and embryonic death occurred by E10. The most prominent defects in these mice were lack of neural fold fusion, attenuation of cranial and spinal nerve development, and importantly, from our perspective, abnormal cardiogenesis with severely reduced development of endocardial structures and an akinetic myocardium. Fibroblasts isolated from mutant embryos showed reduced matrix adhesion and a 2-fold higher migration rate compared with wild-type cells, further amplifying the importance of Vcl in cell-matrix adhesion. This study clearly showed that Vcl was crucial for normal embryonic development, likely due to its role in the regulation of cell-matrix adhesion and cell locomotion.

Vinculin and the Heart: Lessons From Genetically Manipulated Mouse Models

Although homozygous global inactivation of the murine *Vcl* gene caused embryonic lethality[54] with embryos displaying a thin-walled myocardium, hemizygous knock-out (KO) mice ($Vcl^{+/-}$) developed normally and were indistinguishable from wild type ($Vcl^{+/+}$). We hypothesized that the reduced Vcl/MVcl expression in the $Vcl^{+/-}$ heart would lead to impaired cardiac function.[55] With cardiac Vcl/MVcl protein levels reduced to less than 50% of wild-type levels in $Vcl^{+/-}$, these $Vcl^{+/-}$ hearts were surprisingly histologically and functionally normal in the basal state. Electrocardiograms (ECGs) showed no difference in heart rate or PR intervals from WT controls, but displayed widened QRS complexes. Sub-cellular distribution of Vcl and its associated proteins Tln, integrin and cadherin in the heterozygous KO hearts revealed only that the localization of cadherin was abnormal. Given these findings we evaluated the expression pattern of the gap junctional protein connexin 43 (Cx43) in $Vcl^{+/-}$ myocardium. Concordant with the abnormal cadherin expression we found widened and disturbed ICDs, visualized by anti-Cx43 immunostaining. Electron microscopic analyses of $Vcl^{+/-}$ hearts showed aberrant myofibril anchorage at the ICD and Z-lines. Since these findings were present in physiologically normal hearts we tested how $Vcl^{+/-}$ mice would tolerate pressure loading produced by transverse aortic constriction (TAC).[18,56] While all animals survived the surgery, 33% of the $Vcl^{+/-}$ TAC mice died spontaneously during the first 3–6 hours post-operatively. The surviving $Vcl^{+/-}$ mice showed appropriate hypertrophic responses, but beginning at 6-weeks post-TAC developed progressive left ventricular dysfunction. While 100% of control mice survived up to 12-weeks post-TAC, only 30% of $Vcl^{+/-}$ TAC mice survived. When $Vcl^{+/-}$ hearts were examined 4-weeks post TAC, when they were still physiologically normal, abnormalities in Z-line structure was detected. This mouse model gave further insight into the role of Vcl in maintaining function in the pressure loaded left ventricle.

To more specifically address if the cardiac phenotype in the global *Vcl* KO mice was caused by primary loss of Vcl from myocytes, we next generated mice where the *Vcl/MVcl* gene could be excised only in cardiac muscle cells.[57] For this purpose we used a knock-in mouse line that expresses Cre recombinase as driven by the native myosin light chain-2 ventricular promoter.[58] This Cre producing line leads to protein reduction only in the perinatal period.[18,59,60] Homozygous floxed *Vcl* mice that also express the cardiac specific-Cre recombinase (termed c*Vcl*KO) were born with expected Mendelian distributions and developed 70% reduction of MVcl protein. By 6 weeks of age sudden death was evident in the c*Vcl*KO with less than 50% of these mice surviving till 12 weeks of age, despite preservation of cardiac function. Ventricular tachycardia was shown to be the cause of this early death, and the c*Vcl*KO hearts showed defective myocardial conduction. c*Vcl*KO mice that survived through the vulnerable period of sudden death all developed dilated cardiomyopathy and died before 6 month of age. Reduced expression of cadherin and β1integrin was seen in Vcl/MVcl deficient cardiac myocytes. Cx43 expression that was found mainly at the ICD in control mice was redistributed to the lateral cell membrane in the KO cells. Ultrastructural analysis of c*Vcl*KO samples obtained from animals that had preserved ventricular function, showed highly serrated ICD structures, with reduced electron-dense staining throughout the ICD, and mitochondria that were loosely arranged and disorganized. These results showed that loss of Vcl disturbs ICD structure, myofibrillar arrangement and mitochondria prior to the onset of myocardial dysfunction. In the adult heart Cx43 usually localizes to the ICD forming a low resistance pathway for propagation of the electrical impulse between cardiac myocytes. The altered Cx43 distribution we found in c*Vcl*KO mice likely provided for an arrhythmogenic substrate which predisposed to sudden death. The alteration of the ICD structure could also have predisposed c*Vcl*Ko mice toward later myocardial dysfunction. Contrasting the approximate 2 year mouse lifespan with the 80-year life expectancy in humans, suggests the phenotype in our cVclKO mice might be akin to one that would arise in a human teenager. The findings in our mouse models emphasize the importance of maintaining normal Vcl expression in the heart.

Vinculin and Heart: Lessons From Man

Mutations in a large number of genes have now been associated with the development of cardiomyopathies in man.[5] Of this set of genes, *MVcl* was found essentially absent in one patient with dilated cardiomyopathy.[61] A more comprehensive study found multiple mutations of *MVcl* associated with kindreds with both dilated and hypertrophic forms of human cardiomyopathy.[62,63] Interestingly a mutation in *Vcl* was also associated with a predisposition towards only hypertrophic cardiomyopathy.[64,65] These studies begin to show the genetic and functional evidence for *Vcl* as a cardiomyopathy gene.

Gene profiling of patients with non-ischemic cardiomyopathy that required left ventricular assist device (LVAD) support was performed and included analysis of changes in *Vcl* transcripts.[66] Of the group that recovered sufficiently to allow explantation of the device and not go on to heart transplantation, *Vcl* transcripts decreased 1.7-fold from time of implantation to explantation, and Vcl protein decreased 4.1-fold. In contrast, the hearts from patients that could not be assisted with the LVAD and still required transplant showed 1.7-fold increased *Vcl* transcript levels. It was suggested that accumulation of membrane associated proteins, including Vcl, might be a compensatory mechanism typical for end stage heart failure independent of the underlying disease.[67]

Chagas cardiomyopathy caused by *Trypanosoma cruzi* infection leads to severe cardiac fibrosis, life-threatening arrhythmias, and cardiac dysfunction.[68] In vivo and in vitro studies showed that Trypanosoma infection disrupts Vcl's localization at the costamere.[69] At all times of infection, Vcl expression was reduced and Vcl was absent from the costameres of infected specimens that also showed irregular alignment of ICDs. Recently, de Melo et al.[70] showed that in addition to Vcl down-regulation in Chagas disease, a substantial reduction of pan-cadherin and β-catenin expression were also seen at the ICD. Combined, these data suggest that the disruption of costameric Vcl, combined with abnormal cadherin-based cell-to-cell contact sites, may predispose to the impaired cardiac function and rhythm disturbances found in Chagas disease.

Talin: Another Costameric Protein With Potential Importance in the Heart

Integrins are cell-surface receptors that bind cells to ECM, signal, and function as mechanotransducers. They have been shown by our group and others to have an important role in normal cardiac homeostasis and in pathologic processes such as hypertrophy.[24,26] Integrins can bind directly or indirectly to many structural and signaling proteins through their cytoplasmic domain. The function of some of these proteins has begun to be characterized in the heart (eg, FAK, integrin-linked kinase, melusin, and Vcl[55,57,60,71–73]) but others, such as Tln, have not. Tln is a large (270-kd, 2540–amino acid) dimeric protein that connects integrins with the actin cytoskeleton (Fig. 1). It is essential for the structural integrity of FAs and therefore for the attachment of the cells to the ECM.[74] In addition to its structural role, Tln modulates the ligand-binding activity of integrins and functions in signal transduction, recruiting signaling proteins like FAK and integrin-linked kinase[4] to FAs.[75] Tln contains a globular N-terminal head domain (approximately 50 kd) and a large flexible C-terminal rod domain (>200 kd). The head contains a FERM domain (4.1 protein, ezrin, radixin, and moesin) with binding sites for β-integrin subunits, F-actin, Wech, H-Ras, layilin, PIPkinase, and FAK. The rod domain contains an additional integrin-binding site, 2 additional actin-binding sites, multiple-binding sites for Vcl, and a binding site for the muscle-specific protein α-synemin.[75]

There are 2 vertebrate Tln genes (*Tln1* and *Tln2*) that generate proteins that are 74% identical. To date, most studies of Tln have not distinguished between the individual isoforms Tln1 and Tln2. Tln1 is ubiquitously expressed while Tln2 expression is more restricted, having dominant expression in heart, brain and skeletal muscle. Many in vitro and in vivo studies, have analyzed the function of Tln1. In vitro studies have shown that Tln1 is required for the assembly of FAs[74] and the regulation of integrin affinity for ECM ligands. *Tln1* null mice have been created and display an embryonic lethal phenotype at E8.5–9, due to gastrulation defects,[76] indicating that *Tln1* is an essential gene and that *Tln2* cannot replace its function in the entire embryo.

Recent studies using genetically manipulated mouse models have begun to analyze the in vivo relevance of Tln1 in specific organs and cell types. For example one study has demonstrated that Tln1 is required for the activation of integrins in platelets,[77] while another has shown that plasma membrane blebbing with loss of membrane association with the cytoskeleton, occurs in megakaryocytes devoid of Tln1.[78] Like its ubiquitous localization in FAs, Tln is also found in muscle-specific integrin complexes such as costameres, ICDs and myotendinous junctions (MTJs).[79–81] Given the similarities between FAs and costameres, Tln likely serves an essential function in muscles cells of all types. This has begun to be shown by another mouse-based study where Tln1 was ablated specifically in skeletal myocytes.[82] This work showed that within skeletal muscle, Tln1 is mainly localized at MTJs. The importance of Tln at this site was then appreciated in that the *Tln1* muscle specific KO mice showed defects in MTJs, but not in the assembly or localization of costameric integrin complexes, containing β1 integrin, Vcl and α-actinin. These results combined with the relatively high expression of Tln2 in other areas of muscle, suggest that Tln2 may in part compensate for loss of skeletal muscle Tln1 in these mice.

To date, few studies have characterized the function of Tln2. Studies in C2C12 skeletal muscle cells have shown that expression levels of Tln2 increase during myoblast differentiation into myotubes, while Tln1 expression remains constant.[83] These authors also found that in rat cardiac myofibrils, Tln2 was localized in costameres and ICDs while Tln1 was not. This information suggests that Tln1 and Tln2 may have unique functions in skeletal and cardiac muscle.

Further studies on Tln have capitalized on a gene trap mouse model which disrupts the *Tln2* gene.[84] In this mouse, insertion of a beta-galactosidase transgene occurs in exon 28 of the *Tln2* gene, producing a chimeric Tln2 protein which expresses AA 1-1295 of the 2540 total AA protein. With this, a large part of the Tln2 rod domain is not produced but the mice are viable and fertile, suggesting that Tln2 is not an essential protein for normal development or basal bodily functions, or that the Tln2 chimeric protein contains the necessary domains or structures to be completely functional.

To emphasize the importance of Tln2, recent work has studied *Tln2* deficient mice. Like the *Tln1* skeletal muscle specific KO mice, the *Tln2* deficient mice also show abnormal MTJ structure[82] although the defects were considerably more severe and at an earlier age with loss of Tln2. This is consistent with the tenet that Tln2 is the major Tln isoform in skeletal muscle.[85] In this same study, mice were also generated with loss of both Tln1 and Tln2 in skeletal muscle. These mice phenocopied the skeletal muscle specific *beta-1* integrin KO mice[86] in that they showed disruption of skeletal muscle fiber cytoskeletal structure, had defects in both myoblast fusion and sarcomere assembly, and died shortly after birth. This data suggests that Tln1 and 2 are both required for intact integrin function during muscle development and growth.

Given these findings in skeletal muscle, we hypothesize that both Tln isoforms likely play essential roles in cardiac muscle development and remodeling and work is underway in our group to directly test this hypothesis. Indeed, work recently published on the mechanosensory protein cardiac ankyrin repeat protein (CARP), a Z-disc component known mostly for its interaction with titin and myopalladin, also suggested that some CARP mutants which lost binding to Tln1 could be linked to familial cardiomyopathy in man.[87]

Conclusions and Future Perspectives

Recent advances have clearly linked alterations in a large number of genes to all forms of cardiomyopathies.[5,88,89] Among the range of causal genes are ones positioned within mechanical sensors of the cardiac myocyte: the Z disk and the costamere.[10,12] Our hope in this brief review was not to provide an exhaustive review of these proteins but to specifically focus on 2 proteins under study in our laboratory that are examples of these types of proteins.

Mutations of *Vcl* and *MVcl* found in patients with cardiomyopathy; as well as the alteration of Vcl expression and localization in Chagas' disease and in the advanced heart failure patient, all underscore the important role of Vcl in the human heart. *Vcl* KO mouse models as we have studied, further emphasize the critical role of Vcl and MVcl in maintenance of cardiac function. Heterozygous global *Vcl* KO mice showed no basal phenotype, but pressure overload of the left ventricle caused premature heart failure and increased their mortality compared to control animals. This model in mice has many parallels to the increased workload sensed by the heart in patients with hypertension. Since *Vcl* and *MVcl* mutations which have been linked to cardiomyopathy in man are heterozygous ones, we suggest that patients harboring these mutations could be further predisposed to developing premature heart failure when they also have other co-morbidities such as elevated blood pressure. The cardiac specific *Vcl* KO mouse model emphasizes the critical role of Vcl and MVcl in the maintenance of cell-to-cell and cell to matrix junctions in that they rapidly develop replacement fibrosis, arrhythmias and dilated cardiomyopathy. The profound phenotype in c*Vcl*Ko mice clearly shows that Vcl is a fundamental structural protein in cardiac myocytes.

Still, despite these advances, there are many questions which remain to be answered about the role of Vcl in heart and heart disease. For example, though in vitro studies have demonstrated that the tail domains of Vcl and MVcl cause differential bundling of actin filaments,[28] extending this biochemical work to the intact organ must be done. Further, it is clear that Vcl is highly expressed in the T-tubular system of the cardiac myocyte, but direct proof of Vcl's role in maintaining physiological ion-channel function is currently unavailable. Similarly, our studies in the c*Vcl*KO myocardium suggest that Vcl/MVcl is instrumental in anchoring contractile filaments to costameres and ICDs, and may also be critical for normal gap junction function, perhaps through Vcl's direct regulation of the ICD.

Less is even known about the function of Tln in the heart and cardiac myocyte. Tln1 and Tln2 share high similarity in sequence and structure, yet much data about their distinct roles in any cell, let alone ones in heart, is not currently available. Still, the few published studies on Tln function in skeletal muscle[82,83] have led us to hypothesize that Tln1 and Tln2 function must clearly be distinct. Tln's role as a mechanical linker and in force generation of non-muscle cells[74] together with its localization at costameres in skeletal and cardiac muscle, suggests that it could be an important mechanotransduction molecule in muscle. As discussed above, this role is supported by the recent study where CARP/Tln1 binding mutants were linked to dilated cardiomyopathy in human patients.[87] Further studies will be necessary, and are ongoing in our own group, to analyze Tln function in the basal and stressed heart, and to clarify the unique role of Tln1 in contrast to Tln2.

Clearly increased understanding of proteins like Vcl and Tln will allow a greater understanding of the molecular basis for cardiomyopathies, increase our ability to screen and identify patients at high risk for profound heart failure or sudden death, and could potentially lead to novel therapies.

REFERENCES

1. Ahmad F, Seidman JG, Seidman CE. The genetic basis for cardiac remodeling. *Annu Rev Genomics Hum Genet*. 2005;6:185–216.
2. Alcalai R, Seidman JG, Seidman CE. Genetic basis of hypertrophic cardiomyopathy: from bench to the clinics. *J Cardiovasc Electrophysiol*. 2008;19:104–110.
3. Ho CY, Seidman CE. A contemporary approach to hypertrophic cardiomyopathy. *Circulation*. 2006;113:e858–e862.
4. Sabatine MS, Seidman JG, Seidman CE. Cardiovascular genomics. *Circulation*. 2006;113:e450–e455.
5. Hershberger RE, Lindenfeld J, Mestroni L, et al. Genetic evaluation of cardiomyopathy—a Heart Failure Society of America practice guideline. *J Card Fail*. 2009;15:83–97.
6. Maron BJ, Towbin JA, Thiene G, et al. 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*. 2006;113:1807–1816.
7. Ervasti JM. Costameres: the Achilles' heel of herculean muscle. *J Biol Chem*. 2003;278:13591–13594.
8. Danowski BA, Imanaka-Yoshida K, Sanger JM, et al. Costameres are sites of force transmission to the substratum in adult rat cardiomyocytes. *J Cell Biol*. 1992;118:1411–1420.
9. Mansour H, de Tombe PP, Samarel AM, et al. Restoration of resting sarcomere length after uniaxial static strain is regulated by protein kinase Cepsilon and focal adhesion kinase. *Circ Res*. 2004;94:642–649.
10. Hoshijima M. Mechanical stress-strain sensors embedded in cardiac cytoskeleton: Z disk, titin, and associated structures. *Am J Physiol Heart Circ Physiol*. 2006;290:H1313–H1325.
11. Lapidos KA, Kakkar R, McNally EM. The dystrophin glycoprotein complex: signaling strength and integrity for the sarcolemma. *Circ Res*. 2004;94:1023–1031.
12. Cox L, Umans L, Cornelis F, et al. A broken heart: a stretch too far: an overview of mouse models with mutations in stretch-sensor components. *Int J Cardiol*. 2008;131:33–44.
13. Quinlan JG, Hahn HS, Wong BL, et al. Evolution of the mdx mouse cardiomyopathy: physiological and morphological findings. *Neuromuscul Disord*. 2004;14:491–496.
14. Durbeej M, Campbell KP. Muscular dystrophies involving the dystrophin-glycoprotein complex: an overview of current mouse models. *Curr Opin Genet Dev*. 2002;12:349–361.
15. Ervasti JM, Sonnemann KJ. Biology of the striated muscle dystrophin-glycoprotein complex. *Int Rev Cytol*. 2008;265:191–225.
16. Ingber DE. Cellular mechanotransduction: putting all the pieces together again. *FASEB J*. 2006;20:811–827.
17. Ross RS, Pham C, Shai SY, et al. Beta1 integrins participate in the hypertrophic response of rat ventricular myocytes. *Circ Res*. 1998;82:1160–1172.
18. Shai SY, Harpf AE, Babbitt CJ, et al. Cardiac myocyte-specific excision of the beta1 integrin gene results in myocardial fibrosis and cardiac failure. *Circ Res*. 2002;90:458–464.
19. Keller RS, Shai SY, Babbitt CJ, et al. Disruption of integrin function in the murine myocardium leads to perinatal lethality, fibrosis, and abnormal cardiac performance. *Am J Pathol*. 2001;158:1079–1090.
20. Valencik ML, Keller RS, Loftus JC, et al. A lethal perinatal cardiac phenotype resulting from altered integrin function in cardiomyocytes. *J Card Fail*. 2002;8:262–272.
21. Valencik ML, Zhang D, Punske B, et al. Integrin activation in the heart: a link between electrical and contractile dysfunction? *Circ Res*. 2006;99:1403–1410.
22. Legate KR, Wickstrom SA, Fassler R. Genetic and cell biological analysis of integrin outside-in signaling. *Genes Dev*. 2009;23:397–418.
23. Wiesner S, Legate KR, Fassler R. Integrin-actin interactions. *Cell Mol Life Sci*. 2005;62:1081–1099.
24. Ross RS, Borg TK. Integrins and the myocardium. *Circ Res*. 2001;88:1112–1119.
25. Samarel AM. Costameres, focal adhesions, and cardiomyocyte mechanotransduction. *Am J Physiol Heart Circ Physiol*. 2005;289: H2291–H2301.
26. Brancaccio M, Hirsch E, Notte A, et al. Integrin signalling: the tug-of-war in heart hypertrophy. *Cardiovasc Res*. 2006;70:422–433.
27. Belkin AM, Ornatsky OI, Kabakov AE, et al. 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*. 1988;263:6631–6635.
28. Rudiger M, Korneeva N, Schwienbacher C, et al. Differential actin organization by vinculin isoforms: implications for cell type-specific microfilament anchorage. *FEBS Lett*. 1998;431:49–54.
29. Lu MH, DiLullo C, Schultheiss T, et al. The vinculin/sarcomeric-alpha-actinin/alpha-actin nexus in cultured cardiac myocytes. *J Cell Biol*. 1992;117:1007–1022.
30. Jockusch BM, Isenberg G. Interaction of alpha-actinin and vinculin with actin: opposite effects on filament network formation. *Proc Natl Acad Sci U S A*. 1981;78:3005–3009.
31. Burridge K, Mangeat P. An interaction between vinculin and talin. *Nature*. 1984;308:744–746.
32. Hazan RB, Kang L, Roe S, et al. Vinculin is associated with the E-cadherin adhesion complex. *J Biol Chem*. 1997;272:32448–32453.
33. Weiss EE, Kroemker M, Rudiger AH, et al. Vinculin is part of the cadherin-catenin junctional complex: complex formation between alpha-catenin and vinculin. *J Cell Biol*. 1998;141:755–764.

34. 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 U S A*. 1983;80:1008–1012.
35. Ziegler WH, Gingras AR, Critchley DR, et al. Integrin connections to the cytoskeleton through talin and vinculin. *Biochem Soc Trans*. 2008;36:235–239.
36. Demali KA, Burridge K. Coupling membrane protrusion and cell adhesion. *J Cell Sci*. 2003;116:2389–2397.
37. Perriard JC, Hirschy A, Ehler E. Dilated cardiomyopathy: a disease of the intercalated disc? *Trends Cardiovasc Med*. 2003;13:30–38.
38. Bakolitsa C, de Pereda JM, Bagshaw CR, et al. Crystal structure of the vinculin tail suggests a pathway for activation. *Cell*. 1999;99: 603–613.
39. Bakolitsa C, Cohen DM, Bankston LA, et al. Structural basis for vinculin activation at sites of cell adhesion. *Nature*. 2004;430:583–586.
40. Borgon RA, Vonrhein C, Bricogne G, et al. Crystal structure of human vinculin. *Structure*. 2004;12:1189–1197.
41. Johnson RP, Craig SW. An intramolecular association between the head and tail domains of vinculin modulates talin binding. *J Biol Chem*. 1994;269:12611–12619.
42. Weekes J, Barry ST, Critchley DR. Acidic phospholipids inhibit the intramolecular association between the N- and C-terminal regions of vinculin, exposing actin-binding and protein kinase C phosphorylation sites. *Biochem J*. 1996;314(pt 3):827–832.
43. Gilmore AP, Burridge K. Regulation of vinculin binding to talin and actin by phosphatidyl-inositol-4-5-bisphosphate. *Nature*. 1996;381: 531–535.
44. Izard T, Evans G, Borgon RA, et al. Vinculin activation by talin through helical bundle conversion. *Nature*. 2004;427:171–175.
45. Bois PR, O'Hara BP, Nietlispach D, et al. The vinculin binding sites of talin and alpha-actinin are sufficient to activate vinculin. *J Biol Chem*. 2006;281:7228–7236.
46. Ziegler WH, Liddington RC, Critchley DR. The structure and regulation of vinculin. *Trends Cell Biol*. 2006;16:453–460.
47. Huttelmaier S, Illenberger S, Grosheva I, et al. Raver1, a dual compartment protein, is a ligand for PTB/hnRNPI and microfilament attachment proteins. *J Cell Biol*. 2001;155:775–786.
48. Huttelmaier S, Zenklusen D, Lederer M, et al. Spatial regulation of beta-actin translation by Src-dependent phosphorylation of ZBP1. *Nature*. 2005;438:512–515.
49. Samuels M, Ezzell RM, Cardozo TJ, et al. Expression of chicken vinculin complements the adhesion-defective phenotype of a mutant mouse F9 embryonal carcinoma cell. *J Cell Biol*. 1993;121:909–921.
50. Goldmann WH, Schindl M, Cardozo TJ, et al. Motility of vinculin-deficient F9 embryonic carcinoma cells analyzed by video, laser confocal, and reflection interference contrast microscopy. *Exp Cell Res*. 1995;221:311–319.
51. Ezzell RM, Goldmann WH, Wang N, et al. Vinculin promotes cell spreading by mechanically coupling integrins to the cytoskeleton. *Exp Cell Res*. 1997;231:14–26.
52. Saunders RM, Holt MR, Jennings L, et al. Role of vinculin in regulating focal adhesion turnover. *Eur J Cell Biol*. 2006;85:487–500.
53. Sharp WW, Simpson DG, Borg TK, et al. Mechanical forces regulate focal adhesion and costamere assembly in cardiac myocytes. *Am J Physiol*. 1997;273:H546–H556.
54. Xu W, Baribault H, Adamson ED. Vinculin knockout results in heart and brain defects during embryonic development. *Development*. 1998;125:327–337.
55. Zemljic-Harpf AE, Ponrartana S, Avalos RT, et al. Heterozygous inactivation of the vinculin gene predisposes to stress-induced cardiomyopathy. *Am J Pathol*. 2004;165:1033–1044.
56. Rockman HA, Ross RS, Harris AN, et al. Segregation of atrial-specific and inducible expression of an atrial natriuretic factor transgene in an in vivo murine model of cardiac hypertrophy. *Proc Natl Acad Sci U S A*. 1991;88:8277–8281.
57. Zemljic-Harpf AE, Miller JC, Henderson SA, et al. Cardiac-myocyte-specific excision of the vinculin gene disrupts cellular junctions, causing sudden death or dilated cardiomyopathy. *Mol Cell Biol*. 2007;27:7522–7537.
58. Chen J, Kubalak SW, Minamisawa S, et al. Selective requirement of myosin light chain 2v in embryonic heart function. *J Biol Chem*. 1998;273:1252–1256.
59. Crone SA, Zhao YY, Fan L, et al. ErbB2 is essential in the prevention of dilated cardiomyopathy. *Nat Med*. 2002;8:459–465.
60. Peng X, Kraus MS, Wei H, et al. Inactivation of focal adhesion kinase in cardiomyocytes promotes eccentric cardiac hypertrophy and fibrosis in mice. *J Clin Invest*. 2006;116:217–227.
61. Maeda M, Holder E, Lowes B, et al. Dilated cardiomyopathy associated with deficiency of the cytoskeletal protein metavinculin. *Circulation*. 1997;95:17–20.
62. Vasile VC, Will ML, Ommen SR, et al. Identification of a metavinculin missense mutation, R975W, associated with both hypertrophic and dilated cardiomyopathy. *Mol Genet Metab*. 2006;87:169–174.
63. Olson TM, Illenberger S, Kishimoto NY, et al. Metavinculin mutations alter actin interaction in dilated cardiomyopathy. *Circulation*. 2002;105:431–437.
64. Vasile VC, Edwards WD, Ommen SR, et al. Obstructive hypertrophic cardiomyopathy is associated with reduced expression of vinculin in the intercalated disc. *Biochem Biophys Res Commun*. 2006;349:709–715.
65. Vasile VC, Ommen SR, Edwards WD, et al. A missense mutation in a ubiquitously expressed protein, vinculin, confers susceptibility to hypertrophic cardiomyopathy. *Biochem Biophys Res Commun*. 2006;345:998–1003.
66. Birks EJ, Hall JL, Barton PJ, et al. Gene profiling changes in cytoskeletal proteins during clinical recovery after left ventricular-assist device support. *Circulation*. 2005;112:I57–I64.
67. Hein S, Kostin S, Heling A, et al. The role of the cytoskeleton in heart failure. *Cardiovasc Res*. 2000;45:273–278.
68. Marin-Neto JA, Cunha-Neto E, Maciel BC, et al. Pathogenesis of chronic Chagas heart disease. *Circulation*. 2007;115:1109–1123.
69. Melo TG, Almeida DS, de Meirelles MN, et al. Trypanosoma cruzi infection disrupts vinculin costameres in cardiomyocytes. *Eur J Cell Biol*. 2004;83:531–540.
70. de Melo TG, Meirelles MN, Pereira MC. Trypanosoma cruzi alters adherens junctions in cardiomyocytes. *Microbes Infect*. 2008;10: 1405–1410.
71. DiMichele LA, Doherty JT, Rojas M, et al. Myocyte-restricted focal adhesion kinase deletion attenuates pressure overload-induced hypertrophy. *Circ Res*. 2006;99:636–645.
72. Brancaccio M, Fratta L, Notte A, et al. Melusin, a muscle-specific integrin beta1-interacting protein, is required to prevent cardiac failure in response to chronic pressure overload. *Nat Med*. 2003;9:68–75.
73. White DE, Coutu P, Shi YF, et al. Targeted ablation of ILK from the murine heart results in dilated cardiomyopathy and spontaneous heart failure. *Genes Dev*. 2006;20:2355–2360.
74. Zhang X, Jiang G, Cai Y, et al. Talin depletion reveals independence of initial cell spreading from integrin activation and traction. *Nat Cell Biol*. 2008;10:1062–1068.
75. Critchley DR. Biochemical and structural properties of the integrin-associated cytoskeletal protein talin. *Annu Rev Biophys*. 2009;38:235–254.
76. Monkley SJ, Zhou XH, Kinston SJ, et al. Disruption of the talin gene arrests mouse development at the gastrulation stage. *Dev Dyn*. 2000;219:560–574.

77. Petrich BG, Fogelstrand P, Partridge AW, et al. The antithrombotic potential of selective blockade of talin-dependent integrin alpha IIb beta 3 (platelet GPIIb-IIIa) activation. *J Clin Invest*. 2007;117:2250–2259.
78. Wang Y, Litvinov RI, Chen X, et al. Loss of PIP5KIgamma, unlike other PIP5KI isoforms, impairs the integrity of the membrane cytoskeleton in murine megakaryocytes. *J Clin Invest*. 2008;118: 812–819.
79. Law DJ, Allen DL, Tidball JG. Talin, vinculin and DRP (utrophin) concentrations are increased at mdx myotendinous junctions following onset of necrosis. *J Cell Sci*. 1994;107(pt 6):1477–1483.
80. Zhang JQ, Elzey B, Williams G, et al. Ultrastructural and biochemical localization of N-RAP at the interface between myofibrils and intercalated disks in the mouse heart. *Biochemistry.* 2001;40: 14898–14906.
81. Anastasi G, Cutroneo G, Gaeta R, et al. Dystrophin-glycoprotein complex and vinculin-talin-integrin system in human adult cardiac muscle. *Int J Mol Med*. 2009;23:149–159.
82. Conti FJ, Felder A, Monkley S, et al. Progressive myopathy and defects in the maintenance of myotendinous junctions in mice that lack talin 1 in skeletal muscle. *Development*. 2008;135:2043–2053.
83. Senetar MA, Moncman CL, McCann RO. Talin2 is induced during striated muscle differentiation and is targeted to stable adhesion complexes in mature muscle. *Cell Motil Cytoskeleton*. 2007;64: 157–173.
84. Chen NT, Lo SH. The N-terminal half of *talin2* is sufficient for mouse development and survival. *Biochem Biophys Res Commun*. 2005;337:670–676.
85. Conti FJ, Monkley SJ, Wood MR, et al. Talin 1 and 2 are required for myoblast fusion, sarcomere assembly and the maintenance of myotendinous junctions. *Development*. 2009;136:3597–3606.
86. Schwander M, Leu M, Stumm M, et al. Beta1 integrins regulate myoblast fusion and sarcomere assembly. *Dev Cell*. 2003;4:673–685.
87. Moulik M, Vatta M, Witt SH, et al. ANKRD1, the gene encoding cardiac ankyrin repeat protein, is a novel dilated cardiomyopathy gene. *J Am Coll Cardiol*. 2009;54:325–333.
88. Paul M, Zumhagen S, Stallmeyer B, et al. Genes causing inherited forms of cardiomyopathies. A current compendium. *Herz*. 2009; 34:98–109.
89. Margulies KB, Bednarik DP, Dries DL. Genomics, transcriptional profiling, and heart failure. *J Am Coll Cardiol*. 2009;53:1752–1759.