

# Chapter 16

## Basic Mechanisms Mediating Cardiomyopathy and Heart Failure in Aging

### Overview

Biological aging represents the major risk factor for the development of heart failure (HF), malignancies, and neurodegenerative diseases. While risk factors such as lifestyle patterns, genetic traits, blood lipid levels, and diabetes can contribute to its development, advancing age remains the most determinant predictor of cardiac disease. Several parameters of left ventricular function may be affected with aging, including increased duration of systole, decreased sympathetic stimulation, and increased left ventricle ejection time, while compliance decreases. In addition, changes in cardiac phenotype with diastolic dysfunction, reduced contractility, left ventricular hypertrophy, and HF, all increase in incidence with age. Given the limited capacity that the heart has for regeneration, reversing or slowing the progression of these abnormalities poses a major challenge. In this chapter, we present a discussion on the molecular and cellular mechanisms involved in the pathogenesis of cardiomyopathies and HF in aging and the potential involvement of specific genes identified as primary mediators of these diseases.

### Introduction

Although our current knowledge of age-associated cardiac pathologies has outpaced our understanding of the basic mechanisms underlying these processes, with the availability of the Human Genome Project (HGP) and an increasing number of animal models, as well as new and exciting molecular technologies, the unraveling of the underlying basic mechanisms of the failing aging heart has already begun.

Changes occurring in the aging heart include decreased  $\beta$ -adrenergic sympathetic responsiveness [1, 2], slowed and delayed early diastolic filling [3, 4], increased vascular stiffness [5, 6], and endothelial dysfunction [7, 8]. Of significance is the fact that the cellular changes of aging are most pronounced in postmitotic organs (e.g., brain and heart) and defects in the structure and function of cardiomyocytes may

be the determinant factors in the overall cardiac aging process, particularly in HF.

With aging, myocytes undergo hypertrophy, and this may be accompanied by intracellular changes, including mitochondrial-derived oxidative stress (OS) that will contribute to the overall cellular aging as well as to ischemia-induced myocardial damage. Following an episode of ischemia and reperfusion (I/R), the aging heart suffers greater damage than the adult heart; however, the occurrence and degree of aging-related defects remain uncertain. Basic mechanisms that have been proposed for cardiac aging are discussed in this chapter including cell senescent, accumulation of reactive oxygen species (ROS), inflammatory changes, decreased  $\alpha$  and  $\beta$ -adrenoreceptors (AR) mediated contractility, increased levels of G-proteins-coupled receptors, impaired intracellular  $\text{Ca}^{2+}$  homeostasis, decreased IGF-1 levels, cellular damage/cell loss, telomerase inactivation, abnormal autophagy, and altered membrane structure and permeability, all of which may lead to abnormal cardiac contractile function and contribute to the development of HF [9, 10].

### Accumulation of Reactive Oxygen Species

The “free radical theory of aging” has drawn great attention in the assessment of cardiac aging. This theory presupposes that in biological systems, reactive oxidatives species (ROS) attack molecules and cause a decline in the function of organ systems, eventually leading to failure and death. All cell types including cardiomyocytes are capable of generating ROS, and the major sources of their production include mitochondria, xanthine oxidases and the NADPH oxidases. Under pathophysiological conditions, ROS levels can increase and cause cellular damage and dysfunction targeting primarily the mitochondria.

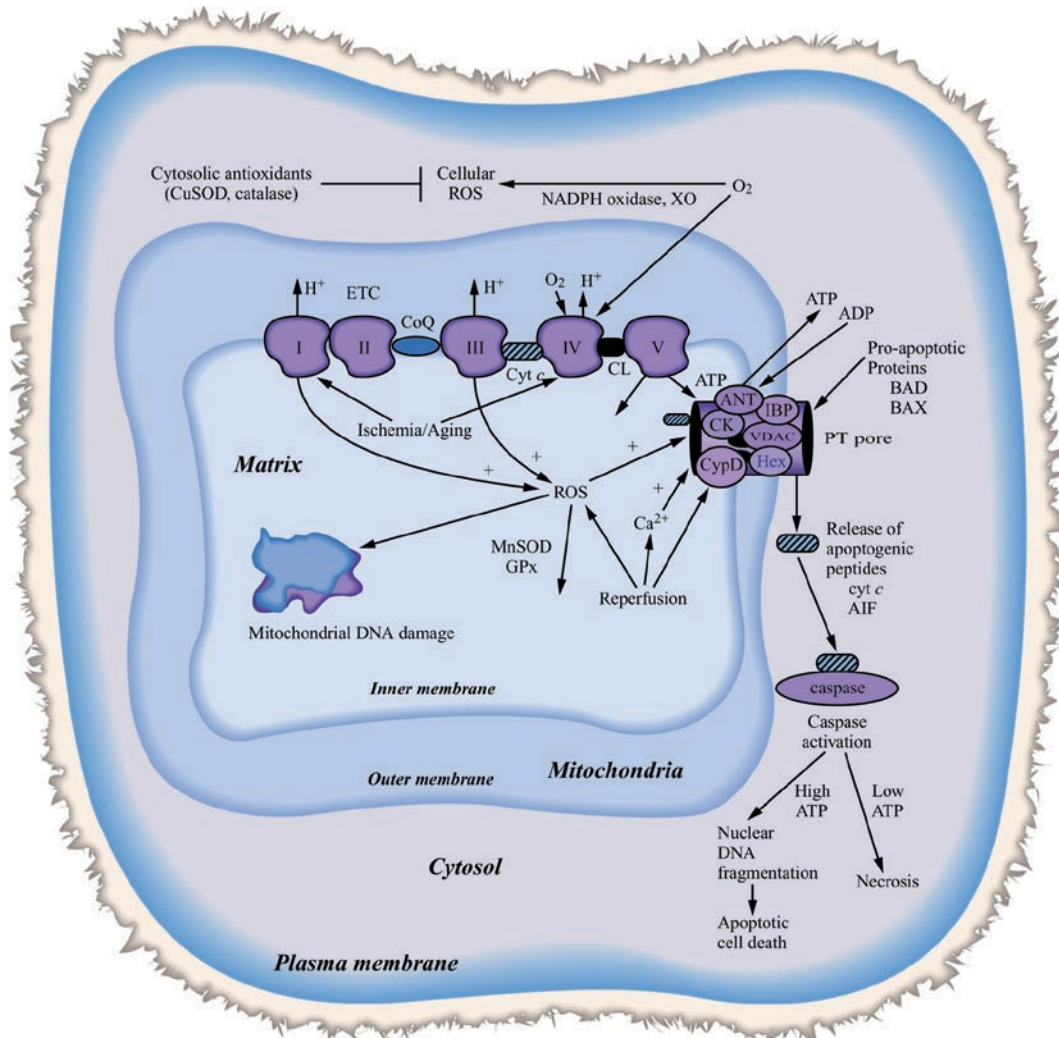
Interestingly, in addition to its damaging effect, ROS play an important role in a number of signal transduction pathways in the cardiomyocyte. Whether the effects of this signaling role are beneficial or harmful may depend upon

the site, source and amount of ROS produced, as well as the overall redox status of the cell. ROS have been implicated in the development of cardiac hypertrophy, cardiomyocyte death by apoptosis, and remodeling of the heart, largely by upregulating proapoptotic proteins and the mitochondrial-dependent pathways. Cardiomyocyte apoptosis has been reported in a variety of cardiovascular diseases, including myocardial infarction, ischemia/reperfusion and HF.

ROS mediates oxidative damage to lipids and proteins in the aging heart, and both myocardial mtDNA and nuclear DNA damage will result in further accumulation of oxidative species and, in particular, mitochondrial DNA damage will accumulate because of its inefficient repair machinery and its close proximity to the sources of ROS (Fig. 16.1). Thus, in the aging failing heart, neutralization of ROS by mitochondrial antioxidants such as superoxide dismutase (SOD), catalase

(CA), glutathione peroxidase (GPx), and glutathione becomes critically important. During aging, mitochondrial dysfunction and ROS generation may also trigger increased apoptosis, with resultant cell loss. This loss in cardiomyocytes may be secondary to mitochondrial dysfunction caused by chronic exposure to ROS, damage to mtDNA (mutations and deletions) and to mitochondrial membranes. While mtDNA damage occurs with aging, mtDNA levels, although decreased in liver and skeletal muscle, are for the most part preserved in the aging heart.

Data from *in vitro* studies indicate that mitochondrial OS and declining mitochondrial energy production can lead to the activation of apoptotic pathways, but whether this also occurs in the *in vivo* aging heart is not clear. While the role and extent of apoptosis in normal myocardial aging is presently unknown, ample evidence of cardiomyocyte apoptosis



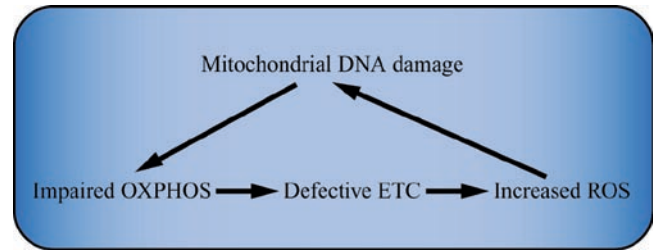
**Fig. 16.1** ROS generation and antioxidant enzymes in the aging heart. Cytosolic pathways of ROS generation involving NADPH oxidase and xanthine oxidase (XO), the cytosolic antioxidant enzymes copper SOD (CuSOD) and catalase are shown, as well as the mitochondrial pathway of

ROS generation (primarily through complex I and III) and mitochondrial antioxidant response featuring MnSOD, GPx and glutathione peroxidase (GPx). Primary mitochondrial targets of ROS including the PT pore, mitochondrial apoptotic pathway and mtDNA are also represented

is supported by studies in the aging rat heart showing the release of cytochrome *c* from mitochondria and decreased levels of Bcl-2 (an antiapoptotic protein), while Bax, a proapoptotic protein, remained unchanged [11, 12].

The occurrence and degree of aging-related defects in mitochondrial OXPHOS remain questionable. Interestingly, aging-related defects have been found in the interfibrillar mitochondria (IFM), in which complex III and IV activity and rate of OXPHOS were decreased, while the subsarcolemmal mitochondrial (SSM) electron transport chain (ETC) activity remained normal [13–15]. The selective alteration of IFM during aging suggests that the consequences of aging-induced mitochondrial dysfunction may be enhanced in specific subcellular regions of the senescent cardiomyocyte. Recent observations suggested that mitochondrial ROS cause OS and impaired mitochondrial function in IFM to a greater degree than in SSM with age, and because of their proximity to myofibrils, IFM are probably the primary source of ATP for myosin ATPases, and therefore OS in IFM may be the culprit for the myocardial dysfunction occurring with aging [16]. It is important to keep in mind that the subfractionation of mitochondria may provide a mixture of organelles of SSM and IFM that may complicate the assessment of the age-related changes in mitochondrial oxidant production and OS. Therefore, further studies in this area are needed.

In early myocardial reperfusion, a burst of ROS occurs in association with changes in mitochondria (e.g., PT pore opening) and myocardial injury. The source of this ROS generation may be of either mitochondrial or cytoplasmic origin. On the other hand, the source of ROS generated during ischemia (and likely in the early/acute pathway of ischemic preconditioning) involves more distinctively the mitochondrial ETC and may be different than the source of ROS generated in early reperfusion [17, 18]. OS also appears to participate in the generation of large-scale myocardial mtDNA deletions as demonstrated in pacing-induced cardiac failure [19], as well as in studies of ameroid constriction-mediated myocardial ischemia in the dog [20]. Moreover, neonatal cardiac myocytes treated with tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) showed a significant increase in ROS levels, and this is accompanied by an overall decline in mtDNA copy number and decreased complex III activity [21]. These findings suggest that the TNF- $\alpha$ -mediated decline in mtDNA copy number might result from an increase in mtDNA deletions. Thus, in aging and in the aging failing heart, accumulation of ROS initiates a vicious circle following somatic mtDNA damage as shown in Fig. 16.2. During aging and after myocardial infarction, ROS-induced mtDNA damage, resulting in respiratory complex enzyme dysfunction, contributes to the progression of left ventricular (LV) remodeling. In a murine model of MI and remodeling created by ligation of the left anterior descending coronary artery, increased ROS production (e.g.,  $\text{OH}^\cdot$  level) was shown in association with decreased levels of mtDNA and



**Fig. 16.2** Increased accumulation of ROS initiates a vicious circle following somatic mtDNA damage

ETC activities, suggesting mitochondrial dysfunction [22]. Significantly, the chronic release of ROS has been linked to the development of left ventricular hypertrophy and advanced HF. As noted in previous chapters, chronic ROS generation can derive both from mitochondria and from nonmitochondrial NADPH oxidase that in endothelial cells is activated by cytokines, neurohormones, and growth factors (e.g., angiotensin II, norepinephrine, TNF- $\alpha$ ) [23, 24]. Furthermore, changes in the cardiac phenotype can be driven by redox-sensitive gene expression, in this way ROS may act as potent intracellular second messengers.

NADPH oxidase plays a prominent role in the hypertrophic signaling pathway [25–27] in cardiac myocytes, and NADPH oxidase activity is significantly increased in the failing myocardium [28]. Interestingly, statins (i.e., 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors) by modulating the ROS-generating activity of NADPH oxidase can inhibit cardiac hypertrophy by cholesterol-independent mechanisms [29, 30]. Also, statins block the isoprenylation and activation of members of the Rho guanosine triphosphatase (GTPase) family such as Rac1, an essential component of NADPH oxidase. Thus, blocking the ROS production with statins may be beneficial to the aging patients with myocardial hypertrophy and chronic HF.

Cardiac overexpression of heavy metal-scavenging antioxidant metallothionein prolongs life span, alleviates aging-associated cardiac contractile dysfunction, insulin insensitivity, and mitochondrial damage [31]. A recent study tested the hypothesis that catalase, an enzyme which detoxifies  $\text{H}_2\text{O}_2$ , might interfere with cardiac aging [32]. Contractile and intracellular  $\text{Ca}^{2+}$  properties were evaluated in cardiomyocytes from young and old FVB (inbred strains of mice that carry the Fv1b allele for sensitivity to the B strain of Friend leukaemia virus) and transgenic mice with cardiac overexpression of catalase. Contractile indices analyzed included peak shortening (PS), time-to-90% PS (TPS90), time-to-90% relengthening (TR90), half-width duration (HWD), maximal velocity of shortening/relengthening ( $\pm dL/dt$ ), and intracellular  $\text{Ca}^{2+}$  levels or decay rate. Levels of advanced glycation endproduct (AGE),  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCX), sarco(endo)plasmic reticulum  $\text{Ca}^{2+}$ -ATPase (SERCA2a), phospholamban (PLB), myosin



heavy chain (MHC), membrane  $\text{Ca}^{2+}$  and  $\text{K}^{+}$  channels were measured by Western blotting. Catalase transgene prolonged survival while did not alter myocyte function by itself. Aging depressed  $\pm dL/dt$ , prolonged HWD, TR90 and intracellular  $\text{Ca}^{2+}$  decay without affecting other indices in FVB myocytes. Aged FVB myocytes exhibited a steeper decline in PS in response to elevated stimulus or a dampened rise in PS in response to elevated extracellular  $\text{Ca}^{2+}$  levels. Aging-induced defects were attenuated by catalase. AGE level was elevated in aged FVB compared with young FVB mice, which was reduced by catalase. Expression of SERCA2a, NCX and  $\text{Kv1.2 K}^{+}$  channel was significantly reduced although levels of PLB, L-type  $\text{Ca}^{2+}$  channel dihydropyridine receptor and  $\beta$ -MHC isozyme remained unchanged in aged FVB hearts. Catalase restored NCX and  $\text{Kv1.2 K}^{+}$  channel but not SERCA2a level in the aged mice. These data suggest that catalase protects the cardiomyocytes from aging-induced contractile dysfunction possibly via improved intracellular  $\text{Ca}^{2+}$  handling. By catalyzing conversion of  $\text{H}_2\text{O}_2$  to oxygen and water, catalase would shift redox balance toward antioxidant end, leading to increased myocardial antioxidant capacity, which may offset the detrimental effect of  $\text{H}_2\text{O}_2$  (a model illustrating the triggering stimuli and cellular pathways involved in the onset and progression of HF is shown in Chap. 5) (see Fig. 5.1).

NO also plays a significant role in myocardial OS as demonstrated by Li and associates. Upon examining the role of inducible nitric oxide synthase (iNOS) in aging-related myocardial ischemic injury, as well as its relation to  $\beta$ -AR stimulation, they found that iNOS is upregulated in the aging rat heart [33]. Isolated perfused hearts from young (3–5 months) and aging (24–25 months) rats subjected to 30 min of myocardial ischemia resulted in cardiac dysfunction. Infusion of isoproterenol for 30 min caused a partial recovery of cardiac function in hearts from young rats, receiving either vehicle or 1,400 W (a nonselective iNOS inhibitor). In striking contrast, isoproterenol infusion to hearts from aging animals receiving vehicle failed to improve ischemia-induced cardiodepression and worsened cardiac function, with a significant increase in myocardial NO production, peroxynitrite formation, caspase-3 activation, and creatine kinase release. Therefore,  $\beta$ -AR stimulation interacts with ischemia and triggers a significant increase in myocardial NO production, creates a nitrosative stress, generates toxic peroxynitrite, activates apoptosis, and eventually causes cardiac dysfunction and myocardial injury in the aging heart. Moreover, myocardial NO production, peroxynitrite formation, and caspase-3 activation were attenuated and LV function significantly improved in aging heart treated with the iNOS inhibitor, 1,400 W. Compared to young rat hearts, significant increases in iNOS protein expression, activity, and immunoreactivity were found in the aging heart, confirming that aging induces a phenotypic upregulation of myocardial iNOS and that there is a critical link between iNOS-generated NO production and aging-associated myocardial ischemia.

## Inflammatory Mechanisms/Signaling

Besides ROS, NO and iNOS other molecules and signaling pathways such as inflammatory signaling are actively involved in the aging process. Gathered observations have shown the fundamental role that immunity has in mediating atherosclerosis from initiation through progression and, ultimately, the thrombotic complications of atherosclerosis [34–36]. Increase in markers of inflammation may predict outcomes of patients with acute coronary syndromes, independently of myocardial damage.

Inflammatory markers have been identified as significant independent risk indicators for cardiovascular events including HF. Kritchevsky et al. [37] have examined the role that inflammatory markers play in predicting the incidence of CVD, specifically in older adults. Interestingly, IL-6, TNF- $\alpha$ , and IL-10 levels appear to predict cardiovascular outcomes in adults <65 years. Data on C-reactive protein (CRP) levels were rather inconsistent and appeared to be less reliable in old age than in middle age. In addition, fibrinogen levels have some value in predicting mortality but in a nonspecific manner. The authors indicated that in the elderly, inflammatory markers are nonspecific measures of health and may predict both disability and mortality, even in the absence of clinical CVD.

Interventions designed to prevent CVD through the modulation of inflammation may be helpful in reducing disability and mortality. The role of increased inflammatory markers such as IL-6 and IL-1 $\beta$  as a risk factor in aging, and in the development of MI has also been reported [38]. Analysis of polymorphisms in *IL-6* gene promoter (-174 G>C) revealed that elderly patients with acute coronary syndrome (ACS) carrying *IL-6* -174 GG genotypes exhibited a marked increase in 1 year follow-up mortality rate, suggesting that *IL-6* -174 G  $\rightarrow$  C polymorphisms can be added to the other clinical markers such as CRP serum levels and a history of CAD, useful in identifying elderly male patients at higher risk of death after ACS [39]. In addition, data from the InCHIANTI study suggest that increased levels of serum IL-1 $\beta$  are associated with high risk of congestive HF and angina pectoris [40].

## Adrenergic Receptors in the Aging Heart

The decline in cardiac performance that occurs with aging is in part due to a decrease in  $\alpha$ - and  $\beta$ -AR-mediated contractility. While impairment in  $\beta$ -AR signaling is known to occur in the aging heart, the components of the  $\alpha$ 1-AR signaling cascade that are responsible for the aging-associated deficit in  $\alpha$ 1-AR contractile function have just begun to be identified. To determine that the aged heart has an impaired response to  $\alpha$ 1-adrenergic stimulation, Montagne et al. [41] measured both cardiomyocyte  $\text{Ca}^{2+}$ -transient and cardiac protein kinase C (PKC) activity in young (3 months) and old

Wistar rats (24 months).  $\text{Ca}^{2+}$  transients were obtained under 1 Hz pacing by microfluorimetry of cardiomyocyte loaded with indo-1 and compared during control conditions and after  $\alpha 1$ -adrenergic stimulation (phenylephrine or cirazoline, an  $\alpha 1$ -specific agonist). In addition, the activity of PKC and PKC translocation index were assayed before and after  $\alpha 1$ -adrenergic stimulation. In the young animals, cirazoline induced a significant increase in  $\text{Ca}^{2+}$  transient for up to  $10^{-9}$  M concentration which returned to control values for larger concentrations. In contrast, in the old animals, there was a constant negative effect of cirazoline on the  $\text{Ca}^{2+}$  transient with a significant decrease at  $10^{-6}$  M compared with both baseline and Krebs's solution. In a dose-response curve to phenylephrine, prior experiments showed that the response of  $\text{Ca}^{2+}$  transient was maximal at  $10^{-7}$  M. This concentration induced a significant increase in  $\text{Ca}^{2+}$  transient in the young and a significant decrease in old rats. The same concentration was chosen to perform PKC activity measurements under  $\alpha 1$ -adrenergic stimulation. In the basal state, PKC activity was higher in the older than in the younger animals but was not different in cytosolic fractions; thus, the translocation index was higher in the old group. Following the administration of phenylephrine, translocation of PKC toward the particulate fraction was observed in the young but not in old rats. Taken together, the data showed that cardiac  $\alpha 1$ -adrenoceptor response was impaired in aged hearts and that the negative effect of  $\alpha 1$ -adrenergic stimulation on  $\text{Ca}^{2+}$  transient in cardiomyocytes in the old rats can be related to an absence of  $\alpha 1$ -adrenergic-induced PKC translocation.

Korzick et al. [42] have also measured  $\alpha 1$ -adrenergic stimulation in the senescent rat heart. Cardiac contractility ( $dP/dt$ ) was analyzed using the Langendorff-perfused hearts isolated from 5 month adult and 24 month old aging Wistar rats, following maximal  $\alpha 1$ -AR stimulation with phenylephrine. Upon assessing the subcellular distribution of PKC $\alpha$  and PKC $\epsilon$ , and their respective anchoring proteins RACK1 and RACK2 by Western blotting, they found that the subcellular translocation of PKC $\alpha$  and PKC $\epsilon$ , in response to  $\alpha 1$ -AR stimulation, is disrupted in the aging myocardium. Age-related reductions in RACK1 and RACK2 levels were also observed, suggesting that alterations in PKC-anchoring proteins may contribute to impaired PKC translocation and defective  $\alpha 1$ -AR contraction in the aged rat heart. Interestingly, the investigators also sought to determine whether age-related defects in  $\alpha 1$ -AR contraction could be reversed by chronic exercise training (treadmill) in adult and aged rat [43]. The data revealed that age-related decrease in  $\alpha 1$ -AR contractility in the rat heart can be partially reversed by exercise suggesting that alterations in PKC levels underlie, at least in part, exercise training-induced improvements in  $\alpha 1$ -AR contraction.

Reperfusion of an isolated mammalian heart with a calcium-containing solution after a brief calcium-free perfusion results in irreversible cell damage (the calcium paradox). Activation of the  $\alpha 1$ -AR pathway confers protection against the lethal injury

of the  $\text{Ca}^{2+}$  paradox via PKC-mediated signaling pathways, and this protection is shared by stimuli common with calcium preconditioning [44]. Notwithstanding these findings, the effect of aging on the human sympathetic nervous system remains a controversial issue. At present, interest in this subject has significantly increased, mainly because diverse cardiac pathologies, including essential hypertension, CAD, HF, and dysrhythmias increase with age, and the sympathetic nervous system may be an important pathophysiological component [45]. However, aging does not have an additive effect in the activation of the sympathetic nervous system that occurs in HF; suggesting that other factors such as CAD and MI may impact the increased incidence of HF with aging [46].

### Cardiac G-Protein-Coupled Receptors

Cardiac G-protein-coupled receptors (GPCRs) that function through stimulatory G-protein  $G_{\alpha_s}$ , such as  $\beta 1$ - and  $\beta 2$ -ARs, play a key role in cardiac contractility (see Chap. 8). Several  $G_{\alpha_s}$ -coupled receptors in the heart also activate  $G_{\alpha_i}$ , including  $\beta 2$ -ARs (but not  $\beta 1$ -ARs); PKA-dependent phosphorylation of  $\beta 2$ -AR can shift its coupling preference from  $G_{\alpha_s}$  to  $G_{\alpha_i}$  [47]. Coupling of cardiac  $\beta 2$ -ARs to  $G_{\alpha_i}$  inhibits adenylyl cyclase (AC) and opposes  $\beta 1$ -AR-mediated apoptosis [48]. Studies on advanced HF have shown that  $G_{\alpha_{12}}$  levels increase with age in both human atria [49], and in ventricles of old (24 months) Fischer 344 rats resulting in diminished AC activity [48]. These levels may subsequently increase the receptor-mediated activation of  $G_i$  through multiple GPCRs. Furthermore, increased  $G_i$  activity is likely to have an adverse effect on heart function since  $G_i$ -coupled signaling pathways in the heart reduce both the rate and force of contraction [50].

Investigation of the effects of age on GPCR signaling in human atrial tissue showed that the density of atrial muscarinic acetylcholine receptor (mAChR) increases with age but reaches statistical significance only in patients with diabetes [51]. Interestingly, in elderly subjects of similar ages, those with diabetes have 1.7-fold higher levels of  $G_{\alpha_{12}}$  and twofold higher levels of  $G_{\beta_1}$ . On the other hand, it has been reported that right atrial mAChR density significantly decreased in advanced age [52]. The disparity between these findings could be explained by differences in age between patient groups; one study examined only adults with an age range from 41 to 85 years [51], while the other study group's age ranged from 5 days to 76 years [52]. Analysis of G-protein-coupled receptor kinase (GRK) activity (by *in vitro* rhodopsin phosphorylation) in the right atria from 16 children (mean age  $9 \pm 2$  years) and 17 elderly patients (mean age  $67 \pm 2$  years) without apparent HF and the RA from four patients with end-stage HF showed that in contrast to the failing human heart, in the aging human heart, GRK activity was not increased [54]. These observations

suggest that GRK activity may not have an important role in  $\beta$ -AR desensitization in the aging human heart, but why GRK's regulation is different in the human aging heart than in the failing human heart is not yet completely understood. Leineweber et al. [53] have found that with aging increase in sympathetic activity develops slowly and moderately, since plasma noradrenaline levels (often taken as an indirect index of sympathetic activity) [54] increase continuously at a 10–15% rate per decade due to enhanced spillover of noradrenaline into the circulation [55, 56]. In contrast, in HF, increases in sympathetic activity occur much more rapidly and are more pronounced than in the aging heart [57]. Thus, the time course and intensity of increases in sympathetic activity in the aging and the failing human heart are dissimilar, and this may explain differences in regulation of GRKs in the aging compared to the failing human heart.

To determine whether changes in GRK activity are an early or late occurrence in human HF, and whether  $\beta$ -adrenoceptor blocker treatment is able to influence myocardial GRK activity, Leineweber et al. [58] have measured  $\beta$ -AR density (by  $(-)[(118)I]$ -iodocyanopindolol binding) and GRK activity (by an in vitro rhodopsin phosphorylation assay) in the right atria from patients at different stages of HF treated with and without  $\beta$ -adrenoceptor blockers, as well as in the four chambers of explanted hearts from patients with end-stage HF. Increase in GRK activity was an early and transient event in the course of HF that may be prevented by  $\beta$ -adrenoceptor blocker treatment. It has been reported that in humans, after 50 years of age, atrial mAChR density exhibits an upward trend with age [51] which differs from most animal studies data, which have been less conclusive by either showing unchanged muscarinic receptor levels [59–62], or by indicating decreased mAChR density with age [63].

### ***SERCA and Thyroid Hormone in the Aging Heart***

Myocardial contraction and relaxation are regulated by the concentration of  $Ca^{2+}$  around the contractile elements in cardiomyocytes. After contraction, relaxation is brought about by lowering  $Ca^{2+}$  levels. A major contribution to this process is made by the sarcoplasmic reticulum  $Ca^{2+}$  ATPase (SERCA), a 110-kD transmembrane protein, which pumps cytoplasmic  $Ca^{2+}$  into the sarcoplasmic reticulum (SR) [64]. Three SERCA genes have been identified so far as SERCA1, SERCA2, and SERCA3 encoding six distinct proteins isoforms by alternative splicing [65, 66] with the SERCA2a being the predominant isoform expressed in the heart. Since SERCA2a plays an important role in intracellular  $Ca^{2+}$  hemostasis and cardiac contractility, the abnormal expression of SERCA2a in the senescent heart is likely to have major biochemical and

pathophysiological effects. As a matter of fact, senescent is characterized by reduced myocardial contractility velocity and prolonged relaxation time. These changes might be related to decreased expression and activity of SERCA, which controls the rate of SR calcium uptake during relaxation. The cardiac SERCA pumps  $Ca^{2+}$  from the cytosol back to the SR and is considered an important determinant of intracellular  $Ca^{2+}$  signaling and cardiac contractility. At least in part, the increased susceptibility to HF of the aging heart may be mediated by abnormal  $Ca^{2+}$  handling and decreased expression of the *SERCA* gene [67]. The molecular mechanisms that regulate cardiac *SERCA* expression in aging are still unclear; however, new studies implicated a decreased thyroid hormone (TH) responsiveness in the aging rat heart; in large part, this decrease involves binding of the TH receptor (TR) and retinoid X receptor (RXR) heterodimer to TH-responsive elements (TREs) located in the *SERCA* and cardiac myosin heavy chain (*MHC*) gene promoters. Age-associated changes in the TR and RXR could explain the age-associated changes in *SERCA* and *MHC* expression. Long et al. found no significant myocardial changes in RXR $\alpha$  or RXR $\beta$  mRNA levels in the aging rat heart, although both  $\alpha$ 1 and  $\alpha$ 2 TR mRNA levels decreased significantly between 2 and 6 months of age [68]. During this time period, the mRNA levels for  $\alpha$ -MHC declined by more than half, whereas  $\beta$ -MHC mRNA levels remained unchangingly low. In contrast, between 6 and 24 months, when mRNA levels for  $\beta$ -MHC increased and  $\alpha$ -MHC continued to decrease, there was a significant decline in TR $\beta$ 1 and RXR $\gamma$  mRNA levels accompanied by a reduction in the TR $\beta$ 1 and RXR $\gamma$  protein levels. Taken together, these findings suggest that decline in  $\alpha$ -MHC gene expression may be biphasic and in part due to a decline in  $\alpha$ 1 (and possibly  $\alpha$ 2) TR levels between 2 and 6 months of age, and a decline in TR $\beta$ 1 and RXR $\gamma$  levels at later age.

Aging-mediated down-regulation of MHC and SERCA mediated by myocardial TH/TR signaling-mediated transcriptional control can be reversed with exercise [69]. While the expression of myocardial TR $\alpha$ 1 and TR $\beta$ 1 proteins is significantly lower in sedentary aged rats than in sedentary young rats, their expression is significantly higher in exercise-trained than in sedentary aged rats. Furthermore, the activity of TR DNA binding to the TRE transcriptional regulatory region in the  $\alpha$ -MHC and *SERCA* genes and the myocardial expression of  $\alpha$ -MHC and *SERCA* (both mRNA and protein) were upregulated with exercise training in the aging heart, in association with changes in the myocardial TR protein levels. In addition, plasma 3,3'-triiodothyronine (T3) and TH levels, which decrease with aging [70, 71], are increased after exercise training. The reversal of aging-induced down-regulation of myocardial TR signaling-mediated transcription of MHC and *SERCA* genes by exercise training appears to be related to the cardiac functional improvement observed in trained aged hearts.



Identification of the specific mechanisms contributing to decreased TH signaling in the aging heart may provide novel insights into potential therapies, keeping in mind that in the aging heart decreased TH activity may be a physiological adaptation. In the aging heart, therapies that increase SERCA activity might improve cardiac performance, and  $\text{Ca}^{2+}$  cycling proteins can be targeted to improve cardiac function [72]. Decline in myocardial SERCA content with age may also contribute to the development of impaired function after I/R. Moreover, the ratio of SERCA to either phospholamban or calsequestrin decreased in the senescent human myocardium [73]. Decreased rates of  $\text{Ca}^{2+}$  transport mediated by the SERCA isoform are responsible for the slower sequestration of cytosolic  $\text{Ca}^{2+}$  and consequently prolonged muscle relaxation times in the aging heart. Knyushko et al. [74] found that senescent Fischer 344 rat heart had a 60% decrease in SERCA activity in comparison to that of young adult hearts, and this functional reduction in activity could be attributed in part to both lower abundance of SERCA protein and increased 3-nitrotyrosine modifications of multiple tyrosines within the cardiac SERCA protein. Nitration in the senescent heart was found to increase by more than two nitrotyrosines per  $\text{Ca}^{2+}$ -ATPase, coinciding with the appearance of partially nitrated Tyr(294), Tyr(295), and Tyr(753) residues. In contrast, skeletal muscle SERCA exhibited a homogeneous pattern of nitration, with full site nitration of Tyr(753) in the young, with additional nitration of Tyr(294) and Tyr(295) in the senescent muscle. The nitration of these latter sites correlates with diminished transport function in both types of muscle, suggesting that these sites have a potential role in the down-regulation of ATP utilization by the  $\text{Ca}^{2+}$ -ATPase under conditions of nitrosative stress.

### **Growth Hormone and IGF-1**

IGF-1/GH/IGF-1 receptor system not only plays an important role in determining organism development and lifespan but is in itself affected by age. IGF-1 decreased linearly with age in both sexes, with significantly higher levels in men than women [75]. The decrease in GH-induced IGF-1 secretion in the elderly suggests that resistance to the action of GH may be a secondary contributing factor in the low plasma IGF-1 concentrations [76]. Decreased IGF-1 levels with age may contribute to the increase in cardiac disease found in the elderly, including HF [77]. Findings from the Framingham Heart Study in a prospective, community-based investigation indicated that serum IGF-1 level was inversely related to the risk for HF in the elderly without a previous MI, suggesting that the maintenance of an optimal IGF-1 levels in aged individuals may reduce the risk for HF [78]. In addition, this study revealed that greater levels or production of the catabolic cytokines TNF- $\alpha$  and interleukin 6 were associated with

increased mortality in community-dwelling elderly adults, whereas IGF-1 levels had the opposite effect [79]. In aged animals and humans, the secretion of GH and the response of GH to the administration of GH-releasing hormone (GHRH) are lower than in young adults [80]. In rodents, a twofold increase in GH receptors has been observed with age but this increase fails to compensate for the reduction in GH secretion [81, 82]. Further studies revealed that the apparent size of the GH receptor was not altered with age, whereas the capacity of GH to induce *IGF-1* gene expression and secretion was 40–50% less in old than in young animal [77].

There is considerable literature indicating that GH administration to old animals and humans raises plasma IGF-1 levels and results in increases in skeletal muscle and lean body mass, a decrease in adiposity, increased immune function, improvements in learning and memory, and increases in cardiovascular function. Interestingly, GH can induce improvement in hemodynamic and clinical status in some patients with chronic HF, largely resulting from the ability of GH to increase cardiac mass [83]. However, disappointing results have been reported in patients with DCM undergoing infusion of GH [84]; this could be related to the choice of an incorrect agent (GH instead of IGF-1) and/or failure to selectively target patients with low IGF-1 levels [85]. Recently, in a meta-analysis of clinical studies, Tritos and Danias [86] evaluated the efficacy and safety of recombinant human growth hormone (rhGH) therapy in severe HF. Therapy with rhGH appears to have beneficial clinical effects in HF including improved exercise duration, maximum oxygen consumption, and New York Heart Association class. Also, there was hemodynamic improvement, including increased cardiac output, decreased systemic vascular resistance and improved left ventricular (LV) ejection fraction, with no adverse effects on diastolic function. Most of the beneficial effects were driven by either uncontrolled or longer duration studies. Interestingly, rhGH therapy slightly increased the risk for ventricular dysrhythmias; although this finding was driven by a single small study. This meta-analysis suggests that rhGH therapy may have beneficial effects in cases of HF secondary to LV systolic dysfunction and that the possibility of prodysrhythmia associated with rhGH therapy merits further assessment. Furthermore, larger randomized trials with longer treatment duration are needed to fully elucidate the efficacy and safety of rhGH therapy in human HF.

Pharmacological administration of GH to adults may pose some risks; mice transgenic for GH and acromegalic patients secreting high amounts of GH have premature death [87]. Thus, caution must be used in the use of IGF-1 for treatment of cardiac diseases. Given its potent antiapoptotic role in proliferation, several studies reported the association of high dosage IGF-1 with human cancers [88, 89], in contrast another study found that overexpression of *IGF-1* in animals or the administration of rhIGF-1 does not have a carcinogenic effect [90].

Interestingly, the Klotho protein which functions as a circulating hormone binds to a cell-surface receptor and represses intracellular signals of insulin and IGF-1, stops aging in mice (a subject that will be further discussed later in this chapter). Amelioration of the aging-like phenotypes in Klotho-deficient mice has been observed by disturbing insulin and IGF-1 signaling, suggesting that Klotho-mediated inhibition of insulin and IGF-1 signaling contributes to its antiaging properties [91].

### **Cellular Damage/Cell Loss**

Since cell damage occurs at random in any organ or tissue, including the heart, a population of damaged cells will always coexist with normal cells at any time in the process of aging; an important unknown relates to the number of damaged cells required to impair organ/tissue function. Kirkwood [92] pointed out that there is a significant difference in assessing cell damage in vitro versus in vivo, with cells in culture reaching a limit in their potential for cell division/differentiation, which may not occur in vivo. Therefore, caution is called for regarding the interpretation of data using different methodologies. During aging, there is a significant loss of postmitotic cells, such as cardiac myocytes, potentially triggered by the onset of mitochondrial dysfunction and ROS generation. For instance, in vitro studies of H<sub>2</sub>O<sub>2</sub>-treated cardiomyocytes showed that increased mitochondrial OS and declining mitochondrial energy production lead to the activation of apoptotic pathways [93, 94], but whether this also occurs in the aging heart in vivo is not known. While the role and extent of apoptosis in normal myocardial aging is under considerable debate, evidence of cardiomyocyte apoptosis has been confirmed by data showing that the aging rat heart had significantly elevated levels of cytochrome *c* release from mitochondria, as well as decreased levels of the anti-apoptotic protein Bcl-2, whereas levels of the proapoptotic protein Bax were unchanged [12]. Furthermore, myocytes derived from hearts of old mice displayed increased levels of markers of cell death and senescence, compared to myocytes from younger animals [95]. It is possible that apoptosis, at least to a certain degree in cardiac aging, may be a protective mechanism to get rid of damaged, potentially dangerous cells in a mechanistic effort to tilt the balance toward healthy cells, although, we do not know where this balance is.

According to Uhrborn et al. [96], glioma cells stained for senescence-associated  $\beta$ -galactosidase activity, apparently specific for senescent cells, showed that enlarged cells gave a distinctive positive staining reaction. This senescence phenotype appears to be dependent on the continuous expression of p16INK4A. Thus, induced expression of p16INK4A in these cells reverted their immortal phenotype and caused immedi-

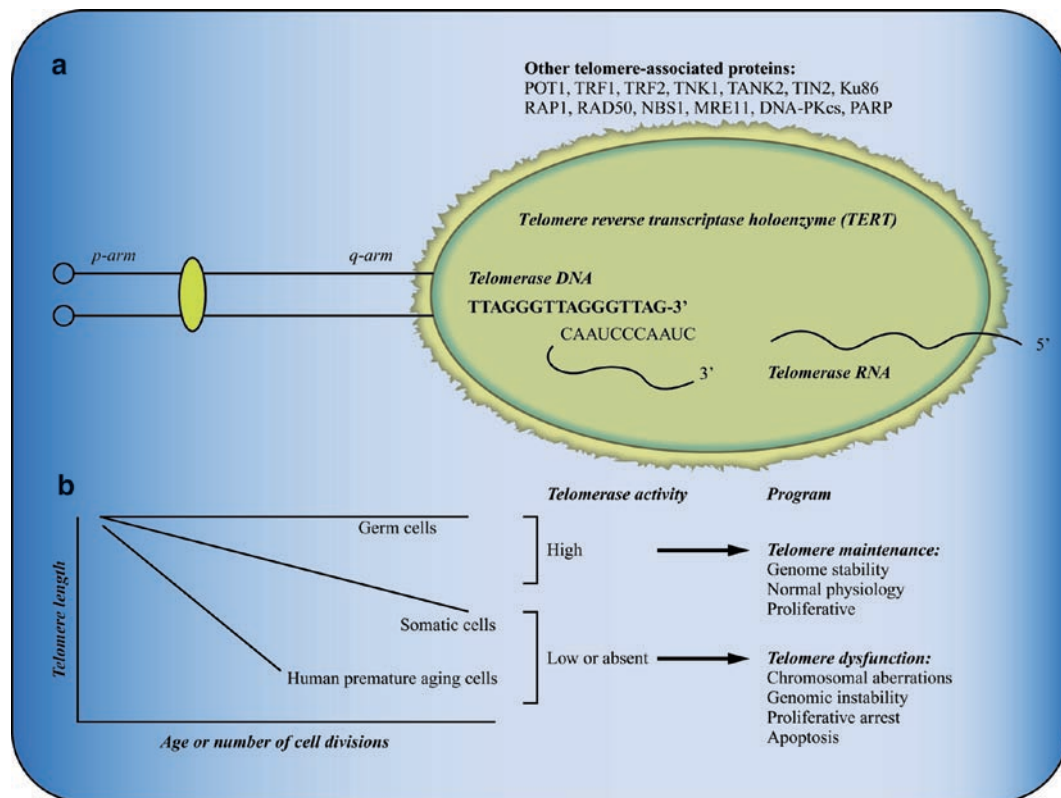
ate cellular senescence. Increased expression of p16INK4A also occurred in aging cardiomyocytes [95]. Proteins implicated in growth arrest and senescence, such as p27Kip1, p53, p16INK4a, and p19ARF, were also present in myocytes of young mice, and their expression increased with age. In addition, DNA damage and myocyte death were found to exceed cell formation in older mice, leading to a decline in the number of myocytes and to HF. This effect did not occur in transgenic mice, in which cardiac stem cells-mediated myocyte regeneration compensated for the extent of cell death and prevented ventricular dysfunction.

### **Telomeres and Telomere-Related Proteins**

In human, there is a remarkable variability in the age of onset and the severity of the manifestations of cardiovascular diseases. Although this cannot be explained by established risk factors, it may be explained by variation in biological age. During aging, telomeres length is progressively reduced in most somatic cells, and after a number of cell cycles, telomere length reaches a critical size, cellular replication stops and the cell becomes senescent (Fig. 16.3). Age-dependent telomere shortening in most somatic cells, including vascular endothelial cells, SMCs, and cardiomyocytes, appears to impair cellular function and the viability of the aged organism. While the association of telomere length and CVD appears likely, whether telomere shortening is a direct cause of the vascular pathology of aging or a consequence is not known [97]. Recently, telomere length has been reported to be shorter in circulating leukocytes of patients with HF compared with age-balanced and gender-balanced control patients, and appears to be related to the severity of disease. Furthermore, telomere length has been found to be incrementally shorter in relation to the presence and extent of atherosclerotic disease manifestations [98]. Notwithstanding, telomere dysfunction and reduction in telomere length has been observed with age in SMCs, endothelial, and white blood cells, and they may be the primary factor in predisposing vascular tissues to atherosclerosis, and also to a decreasing capacity for neovascularization [97]. This attrition of telomere length is most prominent under conditions of high OS, but particularly prevalent in hypertensives, diabetics, and individuals with CAD.

In endothelial cells, glutathione-dependent redox homeostasis plays a central role in the preservation of telomere function [99]. Under conditions of mild chronic OS, the loss of telomere integrity is a major trigger for the onset of premature senescence. Interestingly, antioxidants and statins can delay the replicative senescence of endothelial cells by inhibition of the nuclear export of telomerase reverse transcriptase into the cytosol [100]. Further studies





**Fig. 16.3** Telomeres, telomerase and cell proliferation. (a) Telomeres present at the ends of the chromosomes (one end at the Q arm is shown here) are composed of a tandem repeated sequence in telomeric DNA, a telomerase ribonucleoprotein complex including a catalytic telomerase reverse transcriptase holoenzyme (TERT), an RNA component and additional telomeric proteins (listed on top). (b) Telomere length decreases in aging in somatic cells but not in germ cells. As telomerase

activity is low or absent in most somatic cells, progressive telomere erosion occurs with each mitotic division during normal aging. In germ cells (and tumor cells) containing high telomerase activity, no change in telomere length is seen with aging or progressive divisions. Accelerated telomere attrition is associated with human premature aging. Some of the phenotypic changes associated with telomere length are shown on the *bottom right*

have shown that telomere biology plays a significant role in the functional augmentation of endothelial progenitor cells (EPCs) by statins [101]. These studies found that the *ex vivo* culturing of EPCs leads to premature replicative senescence associated with the “uncapping” and dysfunction of telomeres, and loss of telomere repeat-binding factor (TRF2). In addition, cotreatment of the cultured EPCs with statins delayed their premature senescence, in part by enhancing TRF2 expression at the posttranslational level.

While the ability of EPCs to sustain ischemic tissue and repair may be limited in the aging/senescence heart, estrogens have been shown to accelerate the recovery of the endothelium after vascular injury, significantly increasing telomerase activity [102]. RT-PCR analysis showed that 17 $\beta$ -estradiol administered in a dose-dependent fashion increased levels of the telomerase catalytic subunit (TERT), an effect that was significantly inhibited by pharmacological PI3K blockers (either wortmannin or LY294002). In addition, EPCs treated with 17 $\beta$ -estradiol had significantly enhanced mitogenic potential and release of VEGF protein;

also, EPCs treated with both 17 $\beta$ -estradiol and VEGF were more likely to integrate into the network formation than those treated with VEGF alone. Telomerase inactivation during aging may also be related to the oxidized low-density lipoprotein-accelerated onset of EPC senescence, which leads to the impairment of proliferative capacity and network formation [103].

Estrogen also stimulates NO production in vascular endothelial cells [104], which in turn induces telomerase in these cells [105]. The cardioprotective effects of estrogens via indirect actions on lipoprotein metabolism, and through direct effects on vascular endothelial cells and SMCs are likely to contribute to the lower incidence of CVD observed in premenopausal women compared with men; significantly, women have a decelerated rate of age-dependent telomere attrition over men [106].

In a paper on the relation of telomere biology and cardiovascular disease, Fuster and Andrés [107] reviewed experimental and human studies that linked telomeres and associated proteins to several factors that influence cardiovascular risk

(e.g., estrogens, OS, hypertension, diabetes, and psychological stress), as well as to neovascularization and the pathogenesis of atherosclerosis and heart disease. They identify two still unanswered critical questions: (1) Whether telomere shortening is cause or consequence of cardiovascular disease, and (2) if therapies targeting the telomere may be applicable in treating these disorders (e.g., cell “telomerization” to engineer blood vessels of clinical value for bypass surgery, and to facilitate cell-based myocardial regeneration strategies). Since current research has been mainly focused on the role of telomerase, a suggestion is made that it is of up most importance to investigate whether defects in additional telomere-associated proteins may contribute to the pathogenesis of cardiovascular disease.

Most human somatic cells can undergo only limited replication *in vitro*, and senescence can be triggered when telomeres cannot carry out their normal protective functions. Moreover, senescent human fibroblasts display molecular markers characteristic of cells bearing DNA double-strand breaks and inactivation of DNA damage checkpoint kinases in senescent cells may restore the cell-cycle progression into S phase [108]. This telomere-initiated senescence may reflect a DNA damage checkpoint response that is activated with a direct contribution from dysfunctional telomeres. A high rate of age-dependent telomere attrition has been noted in the human distal abdominal aorta, probably reflecting enhanced cellular turnover rate due to local factors, such as increase in shear wall stress in this vascular segment [109]. These data appear to contradict findings in mice that short telomeres provide a protective effect from diet-induced atherosclerosis [110]. These apparently conflicting findings might be reconciled if cellular damage accumulation imposed by prolonged exposure to cardiovascular risk factors ultimately prevails over protective mechanisms, including telomere shortening [106].

Several observations support the concept that the average telomere length is better maintained in conditions of low OS [111], and selective targeting of antioxidants, directly into the mitochondria, can counteract telomere shortening and increase lifespan in fibroblasts under mild OS [112]. Mitochondrial dysfunction can lead to a loss of cellular proliferative capacity through telomere shortening (Fig. 16.4), and the generation of ROS may signal the nucleus to limit cell proliferation through telomere shortening and telomeres as sensors to damaged mitochondria [113].

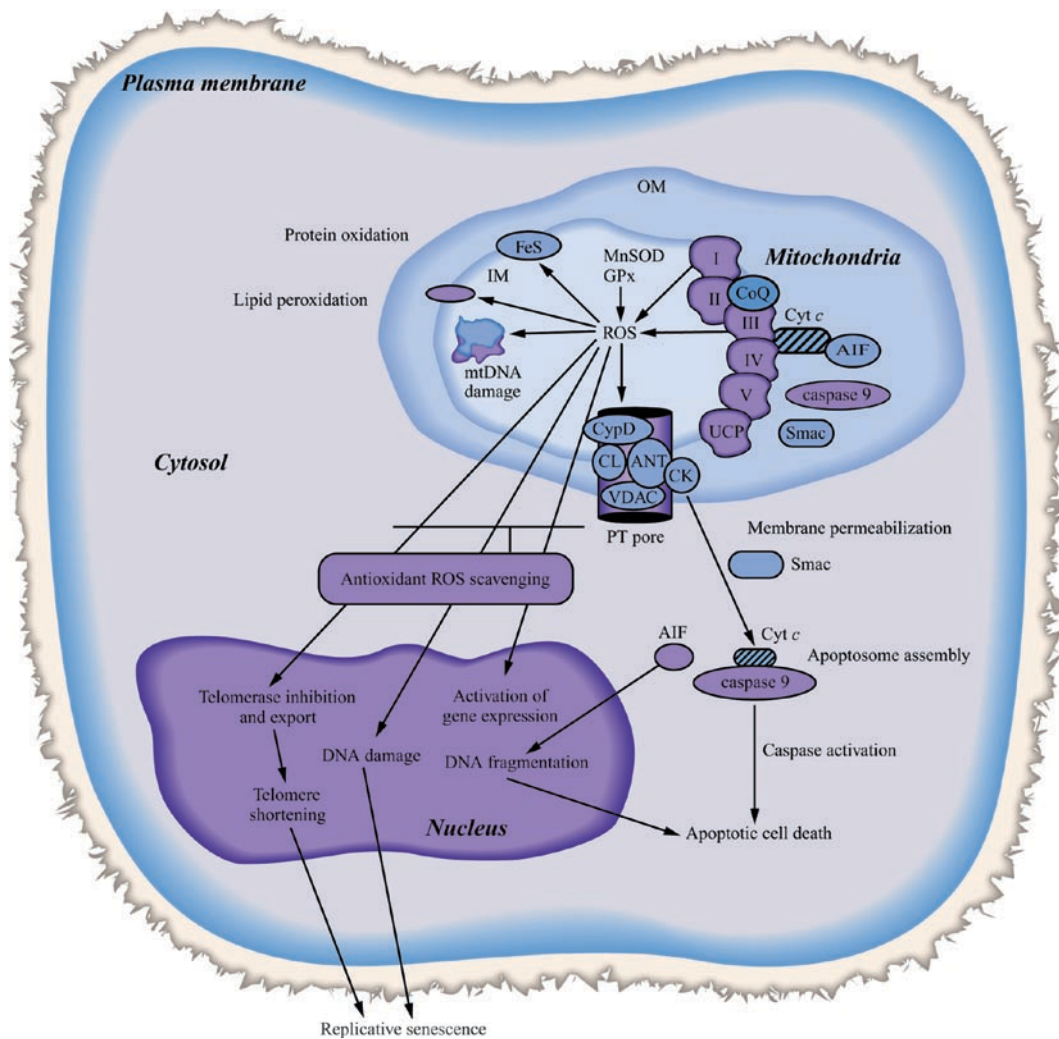
There is increasing evidence that telomere dysfunction is emerging as an important factor in the pathogenesis of human CVDs associated with aging, including hypertension, atherosclerosis, and HF (Fig. 16.5). In the future, further research should also be oriented toward finding the potential role that additional telomere-associated proteins, other than telomerase, play in aging and in HF, and to use these discoveries to develop novel and more effective cardiovascular therapies.

## Autophagy and Cardiac Aging

The process where cells faced with a short supply of nutrients in their extracellular fluid begin to engulf specific, often defective, organelles (e.g., mitochondria) to reuse their components is called autophagy. This process is well conserved in nature from lower eukaryotes to mammals and has been attributed to disparate physiological events including cell death, which mechanism is different from apoptosis. A number of steps are involved in autophagy: (1) formation of a double membrane within the cell; (2) confinement of the material to be degraded into an autophagosome; (3) fusion of the autophagosome with a lysosome, and (4) the enzymatic degradation of the materials. Activated class I phosphatidylinositol 3-kinase and mammalian target of rapamycin (mTOR) inhibit autophagy, while class III phosphatidylinositol 3-kinase acts as a facilitator [114]. Autophagy decreases during the development of myocardial hypertrophy and is enhanced during the regression of hypertrophy. It occurs in many types of cells during development including cardiomyocytes, and in pressure-overloaded animal models cardiomyocyte loss due to autophagy which occurs during the progression from compensated hypertrophy to HF. Moreover, in cardiac diseases associated with aging such as ischemic heart disease and cardiomyopathy, intralysosomal degradation of cells plays an essential role in the renewal of cardiac myocytes; being the interaction of mitochondria and lysosomes in cellular homeostasis of great significance, since both organelles suffer significant age-related alterations in postmitotic cells [115]. Many mitochondria undergo enlargement and structural disorganization, and since lysosomes responsible for mitochondrial turnover experience a loss of function, the rate of total mitochondrial protein turnover declines with age [116]. Coupled mitochondrial and lysosomal defects contribute to irreversible functional impairment and cell death, and similarly mitochondrial interaction with other functional compartments of the cardiac cell (e.g., the ER for Ca<sup>2+</sup> metabolism, peroxisomes for the interchange of antioxidant enzymes essential in the production and decomposition of H<sub>2</sub>O<sub>2</sub>) must be kept in check since defects in communication between these organelles may accelerate the aging process.

Several mechanisms may potentially contribute to the age-related accumulation of damaged mitochondria following initial oxidative injury, including clonal expansion of defective mitochondria, reduction in the number of mitochondria targeted for autophagocytosis (secondary to mitochondrionmegaly or decreased membrane damage associated with decrease mitochondrial respiration), suppressed autophagy because of heavy lipofuscin loading of lysosomes, and decreased efficiency of Lon protease [117].

Abnormal autophagic degradation of damaged macromolecules and organelles, known as biological “garbage,” is also



**Fig. 16.4** Mitochondrial ROS production contributes to telomere-dependent replicative senescence. Production of ROS by aberrant mitochondrial respiratory complexes thought to occur in aging leads to mtDNA and protein damage and lipid peroxidation, PT pore and apoptotic progression (via cytochrome c release, membrane permeabilization, apoptosome formation and nuclear DNA fragmentation). ROS

also affects the nucleus by reducing telomerase activity and increasing its export from the nucleus, causing direct DNA damage and activating specific gene expression – all of which contribute to the signaling of replicative senescence. Also shown is the involvement of antioxidant response both in the mitochondria (e.g., MnSOD and GpX) and in the cytosol which can remove ROS by scavenging free radicals

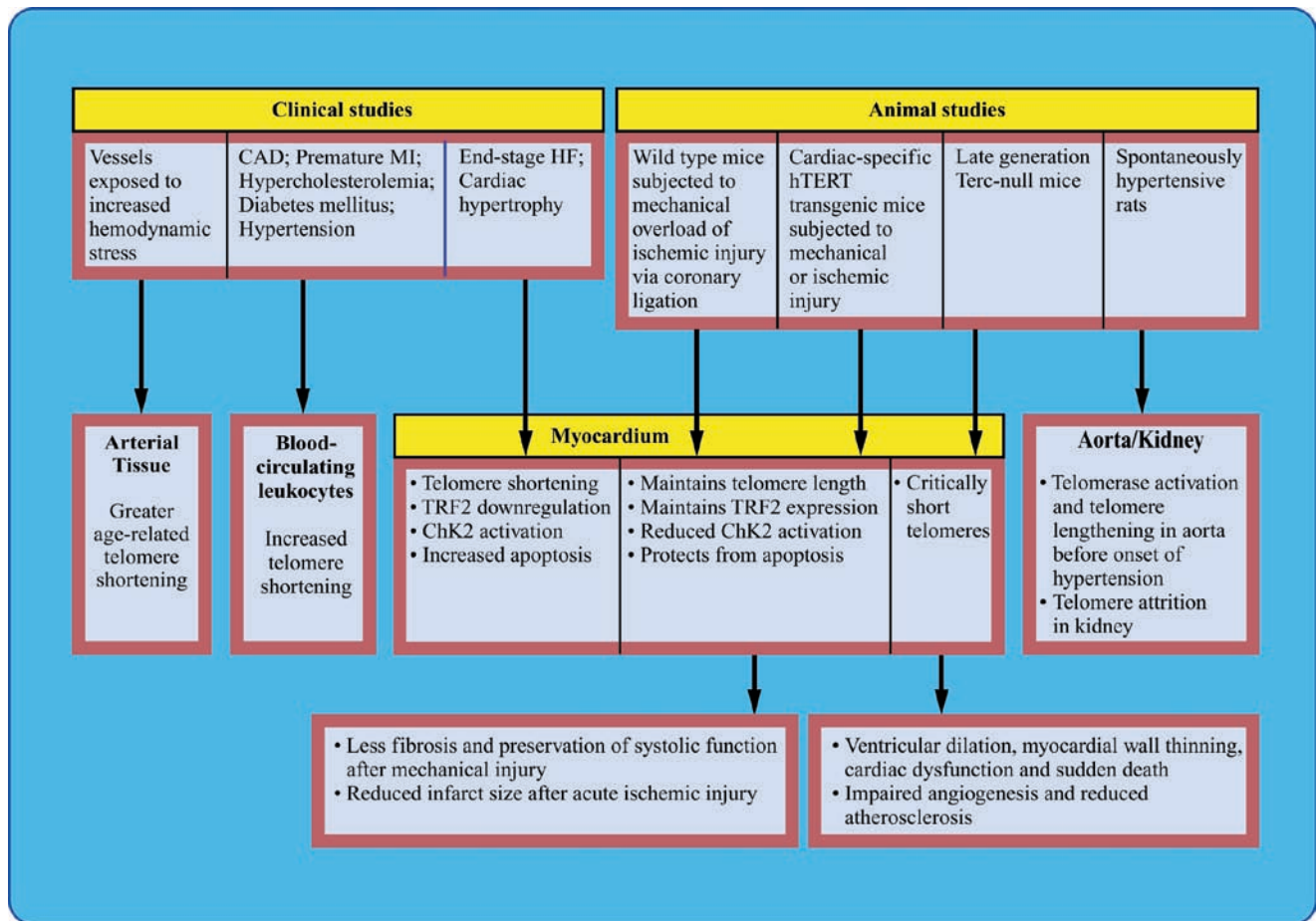
considered an important contributor to aging and the death of postmitotic cells, including cardiomyocytes. Stroikin et al. compared the survival of density-dependent growth-arrested and proliferating human fibroblasts and astrocytes following inhibition of autophagic sequestration with 3-methyladenine (3MA) [118]. Exposure of confluent fibroblast cultures to 3MA for 2 weeks resulted in an increased number of dying cells compared to both untreated confluent cultures and dividing cells with 3MA-inhibited autophagy. Similarly, autophagic degradation was suppressed by the protease inhibitor leupeptin. These findings suggest that lysosomal “garbage” accumulation plays an important role in the aging and death of postmitotic cells, and also support the antiaging role of cell division. Thus, autophagy can be considered an important participant

in the regulation of cellular metabolism, organelle homeostasis and redox equilibrium playing a paramount role in maintaining a normal myocardium.

## Myocardial Remodeling and Aging

To determine the effects of aging on the human myocardium, hearts from individuals (aged 17–90 years) who died from causes other than cardiovascular disease have been studied [119]. The aging process was characterized by a loss of 38 million and 14 million nuclei/year in the left and right ventricles, respectively. This loss in muscle mass was accompanied





**Fig. 16.5** Telomeres and telomerase in both human and animal CVD. Telomere and telomerase phenotype in different tissues of patients with indicated CVD disorders, and of selected animal models of CVD

including both wild-type and genetically modified mice (e.g., cardiac-specific hTERT and Terc-null strains) and in spontaneously hypertensive rats

by a progressive increase in myocyte cell volume per nucleus, resulting in the preservation of ventricular wall thickness. However, the cellular hypertrophic response was unable to maintain normal cardiac mass. Left and right ventricular weights decreased by 0.70 and 0.21 g/year, respectively. Therefore, it was proposed that about one third of the cardiomyocytes are lost from the human heart between the ages of 17 and 90 years [119]. Several studies, however, have challenged the widely held, but unproven paradigm that describes the heart as a postmitotic organ [120]. Recent developments in the field of stem cell biology have led to the recognition that the possibility exists for extrinsic and intrinsic regeneration of myocytes and coronary vessels, leading to the reevaluation of cardiac homeostasis and myocardial aging [121]. A newer paradigm views the adult mammalian heart as composed of nondividing myocytes (primarily terminally differentiated), and a small and continuously renewed subpopulation of cycling myocytes produced by the differentiation of cardiac stem cells. A dynamic balance between myocyte death and the formation of new myocytes by cardiac stem

cells is an important regulator of myocardial maintenance of function and mass from birth to adulthood and very critical in old age. Increasing evidence suggests that numerous pathological or physiological stimuli can activate stem cells to enter the cell cycle and differentiate into new myocytes, and in some cases vasculature which significantly contribute to changes in cardiac output and myocardial mass [122].

Telomere length is a useful marker of these processes. Studies in fetal, neonatal, and senescent (27 month-old) Fischer 344 rats found that while the loss of telomeric DNA was minimal in fetal and neonatal myocytes, telomeric shortening increased with age in a subgroup of myocytes that constituted nearly 20% of the myocyte population, suggesting that this population reflects the most actively dividing myocyte class in the organ. Early studies found in the remaining nondividing rat myocytes, the progressive accumulation of another marker of cellular senescence, senescent associated nuclear protein, p16(INK4) [123].

When cardiomyocytes from aging individuals with age-related HF were compared to myocytes from individuals



with idiopathic DCM, several differences in heart and myocyte phenotype were observed [124]. While aged diseased hearts exhibited moderate hypertrophy and dilation, they also displayed an accumulation of p16INK4a positive primitive stem cells and myocytes. A marked increase in cell death was primarily limited to cells expressing p16INK4a with significant telomeric shortening. Importantly, this finding suggested that stem cells could also be targeted by senescence. While cell multiplication, mitotic index, and telomerase increased in the aging heart, new evidence suggested that regenerative events could not compensate for cell death or prevent telomeric shortening. This study [124] also demonstrated that hearts from subjects with idiopathic DCM had more severe hypertrophy and dilation, more extensive cardiac interstitial fibrosis and tissue inflammatory injury, increased necrosis, and a reduced level of myocytes with p16INK4a labeling. This is consistent with the involvement of p16INK4a with death signals linked to apoptosis in senescent myocardium in contrast to idiopathic DCM, in which myocyte necrosis predominates and myocyte death is largely independent from the expression of the kinase inhibitor p16INK4a. Nonetheless, some important commonalities were also found: while idiopathic DCM had increased necrosis compared to aging tissues, aged diseased hearts and idiopathic DCM subjects had similar levels of myocyte apoptosis. In addition, extensive stem cell death was found in both cases and not in healthy controls, suggesting that mechanisms (e.g., OS) that target stem cells may contribute to the phenotype of cardiac dysfunction in both the aging and cardiomyopathic heart. Torella et al. have identified a population of cardiac stem cells in young and senescent mice that with aging display increased evidence of senescence, i.e., p16ink4a expression, telomere shortening (indicative of reduced telomerase activity), and apoptosis [95]. Interestingly, the effect of murine aging on cardiac stem cells (including the expression of gene products implicated in growth arrest and senescence, such as p27Kip1, p53, p16INK4a, and p19ARF), and on the resulting cardiac dysfunction was remarkably attenuated in aged transgenic mice containing overexpressed IGF-1. It remains to be seen whether similar effects of IGF-1 on preserving stem cell regenerative function would be found in strains with idiopathic DCM.

Recently, Gonzalez et al. [125] reported that chronological age leads to telomeric shortening in cardiac progenitor cell (CPCs), which generate a differentiated progeny that rapidly acquires the senescent phenotype, conditioning organ aging. Attenuation of the IGF-1/IGF-1 receptor and hepatocyte growth factor/c-Met systems mediate the CPC aging, which does not counteract any longer the CPC renin-angiotensin system, resulting in cellular senescence, growth arrest, and apoptosis. However, the senescent heart contains functionally competent CPCs with the properties of stem cells as demonstrated by pulse-chase 5-bromodeoxyuridine-labeling

assay. This subset of telomerase-competent CPCs have long telomeres and, following activation, migrate to the regions of damage, where they generate a population of young cardiomyocytes reversing partly the aging myopathy. Thus, a senescent heart phenotype and HF may be corrected, and this may lead to prolongation of maximum lifespan.

p66(Shc) longevity gene regulates both steady-state and environmental stress-dependent ROS generation and its deletion in mice protects against experimental diabetic glomerulopathy by preventing diabetes-induced OS [126]. The increasing oxygen toxicity that occurs in diabetes mellitus may affect CPCs function resulting in abnormal CPC growth and myocyte formation, which may favor premature myocardial aging and HF. Using a model of insulin-dependent diabetes mellitus, Rota et al. [127] reported that generation ROS leads to telomeric shortening, expression of the senescent associated proteins p53 and p16INK4a, and apoptosis of CPCs, impairing the growth reserve of the heart. Ablation of the p66shc gene prevents these CPCs negative adaptations, interfering with the acquisition of the heart senescent phenotype and the development of HF with diabetes. Low ROS levels activate cell growth, intermediate ROS levels trigger cell apoptosis, and high levels initiate cell necrosis. CPCs replication predominates in diabetic p66shc<sup>-/-</sup>, whereas CPC apoptosis, and myocyte apoptosis and necrosis prevail in diabetic wild type. Expansion of CPCs and developing myocytes preserves cardiac function in diabetic p66shc<sup>-/-</sup>, suggesting that intact CPCs can effectively counteract the impact of uncontrolled diabetes on the heart. The recognition that p66shc conditions the destiny of CPCs raises the possibility that diabetic cardiomyopathy is a stem cell disease, in which abnormalities in CPCs define the life and death of the heart. These data suggest a genetic link between diabetes and ROS and between CPC survival and growth.

Since aging and a number of potential risk factors for coronary artery disease affect the functional activity of the endogenous stem/CPCs, their therapeutic potential application seems rather limited. Furthermore, the aging failing heart may not offer an adequate tissue environment in which cells are infused or injected. To ameliorate this Dimmeler and Leri suggested that pretreatment of cells or the target tissue by small molecules, polymers, growth factors, or a combination thereof may enhance the effect of cell therapy for cardiovascular diseases [128]. However, further research in animal models is necessary before clinical application to aging individual in HF can be carried out.

Besides stem cells and myocytes, another critical component of the aging heart milieu is the cardiac fibroblasts, the predominant cell type in the heart. Fibroblasts activated by various autocrine and paracrine factors, such as angiotensin II (ANGII), aldosterone, endothelins, cytokines, and growth factors likely play a key role in the formation and maintenance

of fibrous tissue by the production of various extracellular-matrix (ECM) proteins, such as collagen, fibronectin, and integrin [129]. Cardiac fibrosis is characterized by excessive accumulation of fibrillar collagen in the extracellular space, in part arising as a loss of cardiomyocytes (replacement fibrosis) and as an interstitial response to various chronic cardiovascular diseases such as hypertension, myocarditis, and severe HF (reactive fibrosis), and is generally considered to be elevated in the aging human heart [1, 130]. Collagen concentration (primarily collagen type 1) and the intermolecular cross-linking of collagen increase with age [131]; however, being a rather complex process, the mechanisms of fibroblast involvement in aging-associated myocardial fibrosis remain undetermined. Surprisingly, both ANGII stimulation of collagen synthesis and fibroblast proliferation are diminished in aging compared to young cardiac fibroblasts [132]. Also, enzymes involved in the degradation of ECM components including the matrix metalloproteinases (MMPs) such as MMP-3, MMP-8, MMP-9, MMP-12, and MMP-14 increased in concert with decreased insoluble collagen in aging mice, suggesting that the accumulated collagen and fibronectin are not attributable to aging-mediated decline in degradation [133, 134]. This suggests that the increased fibrosis and stiffness found in the aging heart must have another mechanism. For example, age-mediated changes in glycation and integrin kinase signaling may contribute to the accumulation of collagen cross-interactions and fiber bundling, which can lead to fibrosis [135, 136]. Increased fibrosis with age leads to increased diastolic stiffness and contractile dysfunction in the heart and its larger vessels, and can reduce the electrical coupling between cardiac myocytes resulting in nonuniform depolarization, conduction delays, and dysrhythmias. It is evident that excessive collagen deposition or pathological fibrosis is an important contributor to LV dysfunction and poor outcome in patients with hypertension, myocardial infarction, and HF.

As previously discussed, HF with preserved LV ejection fraction frequently occurs in the elderly, and this type of HF is known as diastolic heart failure (DHF). Observations on the structural changes of DHF have shown that DHF patients had stiffer cardiomyocytes, as suggested by a higher resting tension ( $F(\text{passive})$ ) at the same sarcomere length. Collagen volume fraction and  $F(\text{passive})$  determined in vivo diastolic LV dysfunction. Correction of this high  $F(\text{passive})$  by protein kinase A (PKA) suggests that reduced phosphorylation of sarcomeric proteins is involved in DHF [137]. Cardiomyocytes of DHF patients had higher  $F(\text{passive})$ , but their total force is comparable to systolic heart failure (SHF). Cardiomyocyte diameter has been found to be higher in DHF [138], but collagen volume fraction was equally elevated in both types of HF, with myofibrillar density lower in SHF. Cardiomyocytes in DHF patients had higher  $F(\text{passive})$ , but their total force was comparable to SHF. After administration of PKA to

cardiomyocytes, the drop in  $F(\text{passive})$  was larger in DHF than in SHF [138]. The data suggest that LV myocardial structure and function differ in SHF and DHF because of distinct cardiomyocyte abnormalities, and underline the separation of HF into two different phenotypes of SHF and DHF.

## Hypertrophic Cardiomyopathy

A number of hypertrophic cardiomyopathy (HCM)-associated alleles have been primarily described in very young individuals showing what is termed early-onset presentation; these phenotypes are often associated with sudden cardiac death and are of significant concern mainly in neonates and young athletes (including specific mutations in  $\beta$ -MHC and cardiac troponin T). Several mutations in fatty acid metabolism (e.g., ACADVL, carnitine transport), and in bioenergetic metabolism (e.g., NDUFB2, COX 15) and a number of mtDNA mutations have also been primarily identified in neonates and children. Interestingly, while the relationship between genotype and phenotype with HCM mutations is often not clear-cut, the distribution of specific mutations in elderly onset disease is often markedly different from specific mutations found in familial early onset HCM [139]. For instance, mutations in *MYBPC3* encoding cardiac myosin binding protein-C, show delayed onset of HCM and induce the disease predominantly in the fifth or sixth decade of life and along with specific troponin I, and  $\alpha$ -myosin heavy chain alleles are more prevalent in elderly onset HCM [140, 141]. Mutations which alter the charge of the encoded amino acid tend to affect patient survival more significantly than those that produce a conservative amino acid change [142]. The milder cardiac phenotype in many of the patients with elderly onset HCM genes is also consistent with the observation that HCM is frequently well tolerated and compatible with normal life expectancy, and it may remain clinically dormant for long periods of time with symptoms and initial diagnosis deferred until late in life [143].

Mutations in the X-linked *GLA* gene encoding the lysosomal enzyme  $\alpha$ -galactosidase A, causing  $\alpha$ -galactosidase A deficiency, is a genetic defect with significant association to late-onset HCM, and leads to an inborn lysosomal storage disorder characterized by pathological intracellular glycosphingolipids deposition. Cardiac involvement consisting of progressive left ventricular hypertrophy is very common and constitutes the most frequent cause of death. Mutations in this gene have been found in up to 6% of men with late-onset HCM, and in 12% of women with late-onset HCM [144, 145].

Of the HCM-causing genes thus far identified (Table 16.1), a large proportion encode protein components of the cardiac sarcomere, which were the first class of HCM genes to be

**Table 16.1** Genes implicated in clinical HCM

Gene	Protein	Function
<i>TNNT2</i>	Cardiac troponin T	Sarcomeric
<i>TTN</i>	Titin	Z disc
<i>MYL3</i>	Essential myosin light chain	Sarcomeric
<i>TNNC1</i>	Cardiac troponin C	Sarcomeric
<i>MYBPC3</i>	Cardiac myosin binding protein C	Sarcomeric
<i>CSRP3</i>	Cardiac muscle LIM protein	Cytoskeletal/Z disc
<i>MYL2</i>	Regulatory myosin light chain	Sarcomeric
<i>MYH7</i>	$\beta$ -Myosin heavy chain	Sarcomeric
<i>ACTC</i>	Cardiac actin	Sarcomeric
<i>TPM1</i>	$\alpha$ -Tropomyosin	Sarcomeric
<i>TNNI3</i>	Cardiac troponin I	Sarcomeric
<i>CAV3</i>	Caveolin-3	Signaling
<i>LAMP2</i>	Lysosome associated membrane protein 2	Lysosome
<i>TCAP</i>	T-Cap (telethonin)	Cytoskeletal/Z disc
<i>MYOZ2</i>	Myozenin 2	Cytoskeletal/Z disc
<i>PRKAG2</i>	AMP-activated protein kinase (regulatory subunit)	Energy sensor
<i>SCO2</i>	COX assembly	Energy metabolism
<i>NDUFV2</i>	Respiratory complex I subunit	Energy metabolism
<i>NDUFS2</i>	Respiratory complex I subunit	Energy metabolism
<i>ANT</i>	Adenine nucleotide transporter/mtDNA maintenance	Energy metabolism
<i>ACADVL</i>	VLCAD activity (Fatty acid oxidation)	Energy metabolism
<i>FRDA</i>	Mitochondrial iron import	Energy metabolism
<i>COX10</i>	COX assembly	Energy metabolism
<i>SLC22A4</i>	Carnitine transporter (OCTN2)	Energy metabolism
<i>COX15</i>	COX assembly	Energy metabolism
<i>GLA</i>	$\alpha$ -Galactosidase	Lysosomal storage

identified, and still remain the most prevalent [146]. Mutations have been found in genes that encode components of the thick filament proteins (i.e.,  $\beta$ -MHC, essential MLC, regulatory MLC, and cMyBP-C), genes that encode thin filament proteins (i.e., cardiac actin, cardiac troponin T, cardiac troponin I, cardiac troponin C, and  $\alpha$ -tropomyosin). HCM mutations have also been found in genes involved in the cytoskeleton including titin, telethonin, myozenin, and cardiac muscle LIM protein. In addition, mutations resulting in HCM have also been reported in genes encoding nonsarcomeric/noncytoskeletal proteins including the  $\gamma$ 2-regulatory subunit of an AMP-activated protein kinase (AMPK) [147], LAMP-2, a lysosome-associated membrane glycoprotein, and caveolin-3, a contributor to the caveolae micro-domains involved in cellular signaling [148]. Another significant subgroup of mutant genes identified in patients with HCM are comprised of genes encoding proteins involved in mitochondrial bioenergetic metabolism. These include subunits of the mitochondrial respiratory chain (NDUFV2, NDUFS2), SCO2 encoding a copper metallochaperone, COX10, and COX15 involved in the assembly of the multisubunit mitochondrial respiratory complex IV enzyme also known as cytochrome *c* oxidase;

genes involved in fatty acid transport and oxidation (SLC22A4, ACADVL) and iron import (FRDA/frataxin); mutations in ANT encoding the adenine nucleotide translocator which also has been recently found to play a role in mtDNA maintenance. Moreover, numerous mutations in mtDNA have also been reported associated with the development of HCM often in conjunction with multisystemic disorders including hypotonia, myopathies, muscle-weakness, lactic acidosis, deafness, ophthalmic disease, and diabetes.

## Dilated Cardiomyopathy

Dilated cardiomyopathy (DCM) is a heterogeneous disease that often overlaps with inflammatory heart disease. Although in the majority of cases the etiology of DCM is unknown, microarray techniques, performed using endomyocardial biopsy specimens, may allow novel insights into the unique disease-specific gene expression that exists in end-stage cardiomyopathy of different etiologies presenting with HF. Importantly, expression profile analysis of DCM may facilitate its early detection and prognostic assessment. Recently, Ruppert et al. [149] characterized different types of DCM employing gene expression profiling of biopsied cardiac tissue with microarrays performed by hybridization of synthesized complementary DNA against a Lab-Arraytor60-combi microarray. One pattern of gene expression was consistent with DCM and inflammatory cardiomyopathy, and another with inflammatory heart disease. Additionally, the microarray data were confirmed by showing that DCM is associated with a reduced myocardial toll-like receptor 9 expression resulting from progressive loss of functional cardiomyocytes. Collectively, these findings demonstrated the utility and validity of microarrays from endomyocardial biopsy specimens to detect subclasses of DCM that do not differ histopathologically, but transcriptionally, from each other. Furthermore, the gene expression profile observed in these patients with DCM suggested ongoing immune activation. Haeidecker et al. have assessed individual risk of new-onset HF by analysis of transcriptome biomarkers using microarrays techniques [150]. From a total of 350 endomyocardial biopsy samples, they were able to identify 180 with idiopathic DCM. Patients with phenotypic extremes in survival were selected: 25 considered to have good prognosis (event-free survival for at least 5 years) and 18 with poor prognosis (events (death, requirement for LVAD, or cardiac transplant) and showing within the first 2 years of presentation clinical HF). Forty-six overexpressed genes were identified in patients with good versus poor prognosis, of which 45 genes were selected by prediction analysis of microarrays for prognosis in a train set ( $n=29$ ) with subsequent validation in test sets ( $n=14$  each). Interestingly, the biomarker performed

with 74% sensitivity and 90% specificity after 50 random partitions. These findings suggest that transcriptomic biomarkers may predict prognosis in patients with new-onset HF from a single endomyocardial biopsy sample. Moreover, and based on these findings, novel therapeutic targets for HF and cardiomyopathy might become possible.

Kittleson et al. [151], also using gene expression analysis of microarrays, have tested the hypothesis that nonischemic (NICM) and ischemic cardiomyopathy (ICM) would have both shared and have distinct differentially expressed genes relative to normal hearts. Comparison of gene expression of 21 NICM and 10 ICM samples with that of six nonfailing (NF) hearts was carried out using Affymetrix U133A Gene Chips and significance analysis of microarrays. Compared with NF, 257 genes were differentially expressed in NICM and 72 genes in ICM. Only 41 genes were shared between the two comparisons, mainly involved in cell growth and signal transduction. Those uniquely expressed in NICM were frequently involved in metabolism, and those in ICM more often had catalytic activity. Novel genes, which were upregulated in NICM but not ICM included angiotensin-converting enzyme-2 (ACE2) suggesting that ACE2 may offer differential therapeutic efficacy in NICM and ICM. In addition, a tumor necrosis factor receptor was downregulated in both NICM and ICM, demonstrating the different signaling pathways involved in the pathophysiology of HF. Thus, transcriptome analysis offers novel insights into the pathogenesis-based therapies in HF management and complements studies using expression-based profiling to diagnose HF of different etiologies. In contrast, Kuner et al. [152] have reported poor separation of ischemic and nonischemic cardiomyopathies by genomic analysis. They analyzed one cDNA and two publicly available high-density oligonucleotide microarray studies comprising a total of 279 end-stage human HF samples. When classifiers identified in a single study were applied to the remaining studies, misclassification rates >25% for ICM and NICM specimens were noted, indicating poor separation of both etiologies. However, data mining of 458 classifier genes that were concordantly identified in at least two of the three data sets points to different biological processes in ICM vs. NICM. Consistent with the underlying ischemia, cytokine signaling pathways and immediate-early response genes were overrepresented in ICM samples, whereas NICM samples displayed a deregulation of cytoskeletal transcripts, genes encoding for the major histocompatibility complex, and antigen processing and presentation pathways, potentially pointing to immunologic processes in NICM. These data suggest that ICM and NICM exhibit substantial heterogeneity at the transcriptomic level. However, prospective studies will be necessary to test whether etiology-specific gene expression patterns are present at earlier disease stages or in subsets of both etiologies.

Other than immune-inflammatory and ischemic etiologies, most often than not, the cause of DCM is unknown and fre-

quently appears to be secondary to mutations in a large number of genes with diverse functions, with a familial inheritance [146]. These mutations may be present at a wide variety of genetic loci that confirm its genetic heterogeneity (Table 16.2). A compounding factor in the development of DCM is age. With aging the incidence of idiopathic DCM increases, males are afflicted at a higher rate than females and elderly patients have a worse prognosis than their younger counterparts with this disease. Moreover, age-related penetrance, i.e., absence of disease manifestations in genotype-positive individuals until after a particular age, and nonpenetrance, has contributed to the underestimation of the prevalence of familial type of this disease [153, 154].

The first gene identified by candidate gene analysis in DCM was cardiac actin. Mutations in five other genes encoding sarcomere proteins have been implicated in DCM (all of which can also cause HCM) including  $\beta$ -MHC, cMyBP-C, cardiac troponin T,  $\alpha$ -tropomyosin, and titin. While a subset of HCM patients develop a dilated phenotype, DCM resulting from sarcomeric gene mutations often arises without previous HCM. Mutations were subsequently described in genes encoding cytoskeletal proteins including desmin,  $\delta$ -sarcoglycan, muscle LIM protein,  $\alpha$ -actinin-2, Cypher/Zasp, Tcap/telethonin and metavinculin, which stabilize the myofibrillar apparatus and link the cytoskeleton to the contractile apparatus. Dystrophin is another critical gene involved in the intracellular cytoskeleton (linking it to the ECM and contributing to intracellular organization, force transduction, and membrane stability), whose mutation can lead to DCM either in association with Duchenne muscular dystrophy or result in an adult-onset X-linked DCM without skeletal myopathy. Furthermore, mutations in gene-products serving critical electrophysiological function in the heart have been associated with DCM in a limited number of cases, including the ABCC9 gene encoding SUR2A, the regulatory subunit of the cardiac  $K_{ATP}$  channel, and the SCN5A gene encoding the cardiac sodium channel, involved in the generation of the action potential. Mutations in the phospholamban (PLN) gene encoding a transmembrane phosphoprotein which by inhibiting the cardiac sarcoplasmic reticular  $Ca^{2+}$ -adenosine triphosphatase (SERCA2a) pump is a critical regulator of calcium cycling, have also been found in individuals with DCM [155–157]. A T116G point mutation, substituting a termination codon for Leu-39 (L39stop), and resulting in loss-of-function mutation (PLN null allele) was identified in two families with hereditary HF; subjects homozygous for L39stop developed DCM and HF, requiring cardiac transplantation [156].

DCM-causing mutations have also been found in proteins which appear to be involved in the maintenance of nuclear integrity as well as playing roles in other nuclear processes including gene transcription, cell cycle regulation, and chromatin remodeling. For example, mutations in the LMNA



**Table 16.2** Genes implicated in familial human dilated cardiomyopathy (DCM)

Gene	Protein	Function	Chromosomal locus	Inher.
<i>ABCC9</i>	SUR2A, regulatory subunit of cardiac K <sub>ATP</sub> channel	Membrane channel	12p12.1	AD
<i>ACTC</i>	Cardiac $\alpha$ -actin	Sarcomeric	15q14	AD
<i>ACTN2</i>	$\alpha$ -actinin-2	Cytoskeletal	1q43	AD
<i>DES</i>	Desmin	Cytoskeletal	2q35	AD
<i>DMD</i>	Dystrophin	Cytoskeletal	Xp21	X-R
<i>FOXD4</i>	Forkhead Box D4	Transcription factor	9p11–q11	Nd
<i>TAZ (G4.5)</i>	Tafazzin	Metabolic	Xq28	X-R
<i>LMNA</i>	Lamins A and C	Nuclear membrane	1p1–q21	AD
<i>CSRP3</i>	muscle LIM protein	Cytoskeletal	11p15	AD
<i>MYBPC3</i>	Cardiac myosin binding protein C	Sarcomeric	11p11	AD
<i>MYH6</i>	$\alpha$ -myosin heavy chain	Sarcomeric	14q12	AD
<i>MYH7</i>	$\beta$ -myosin heavy chain	Sarcomeric	14q12	AD
<i>PLN</i>	Phospholamban	Calcium cycling	6q22.1	AD
<i>SCN5A</i>	Cardiac sodium channel	Membrane channel	3p22–25	AD
<i>SGCD</i>	$\delta$ -sarcoglycan	Cytoskeletal	5q33	AD
<i>STA</i>	Emerin	Nuclear membrane	Xq28	X-R
<i>TCAP</i>	Tcap/telethonin	Cytoskeletal	17q12	AD
<i>TNNC1</i>	Cardiac troponin C	Sarcomeric	3p21.3–3p14.3	AD
<i>TNNI3</i>	Cardiac troponin I	Sarcomeric	19q13.4	AR
<i>TNNT2</i>	Cardiac troponin T	Sarcomeric	1q32	AD
<i>TPM1</i>	$\alpha$ -Tropomyosin	Sarcomeric	15q22	AD
<i>TTN</i>	Titin	Cytoskeletal	2q31	AD
<i>VCL</i>	Metavinculin	Cytoskeletal	10q22–q23	AD
<i>ZASP</i>	Cypher/Zasp	Cytoskeletal	10q22.3–q23.2	AD

AD autosomal dominant; AR autosomal recessive; X-R X-linked recessive; Nd not determined

gene encoding lamin A and the STA gene encoding emerin, both multifunctional nuclear membrane proteins, have been found to lead to DCM, the latter most commonly in association with Emery–Dreifuss muscular dystrophy. A mutation disrupting an extremely highly conserved tryptophan residue in the forkhead domain of FOXD4, a nuclear transcription factor of the forkhead/winged helix box (FOX) gene family has been identified in a pedigree presenting with a complex phenotype including DCM [158].

DCM may be also associated to mutations in proteins involved in energy metabolism, and to specific pathogenic mutations in mtDNA (often in conjunction with multisystemic disorders). Clearly, the wide spectrum of defective intracellular functions that can lead to DCM suggests that multiple pathophysiological mechanisms are likely involved in triggering this disorder, consistent with its frequently heterogeneous presentation. A number of the aforementioned genetic defects are associated with late-onset DCM with higher incidence of presentation in the elderly. In a large-scale mutation analysis of European patients with DCM, carriers of mutations in the  $\beta$  myosin heavy chain (MYH7) gene were significantly older (mean age at diagnosis was 48 years) compared to carriers of mutations in the cardiac T troponin (TNNT2) gene (mean age at diagnosis was 23 years) [159]. Also, several specific DCM-associated mutations are associated with a milder presentation and increased disease presen-

tation with age. While mutations in the MyBP-C have been previously described in association with a favorable clinical course and with late onset HCM, late-onset DCM has been reported in association with an Arg820Gln mutation in the MyBP-C gene [160]. Similarly, carriers of the Arg71Thr mutation in the SGCD gene encoding the cytoskeletal  $\delta$ -sarcoglycan had a relatively mild phenotype and a late onset of DCM [161].

Mutations in the DMD gene have been reported in both familial and sporadic cases of Duchenne (DMD) and Becker (BMD) muscular dystrophies [162, 163]. DMD usually presents in early childhood with progressive skeletal muscle weakness, mainly of the large proximal muscle groups, and loss of ambulation generally by early adolescence; DCM and conduction defects present late in the disease and the majority of patients die in their twenties, most commonly as a result of respiratory failure. Individuals with DMD usually have frameshift or nonsense DMD mutations that result in premature termination of translation, and a reduction or absence of dystrophin; typically, patients with DMD lack any detectable dystrophin expression in their skeletal muscles. In contrast, BMD is a milder, allelic form of DMD with affected males presenting later in life, exhibiting a milder course and displaying a high incidence of cardiac involvement, despite their milder skeletal muscle disease; the most common cause of death in BMD is HF. While clinical

expression occurs primarily in the young adult, expression including severe DCM has also been reported in the older adult (>50 years old) [164, 165]. Subjects with BMD usually have DMD deletions that result in truncation or reduced levels of expression of dystrophin. Skeletal muscle from patients with BMD contains dystrophin of altered size and/or reduced abundance. Interestingly, female carriers of DMD and BMD experience a high incidence of cardiac involvement that progresses with age and manifests primarily as cardiomyopathy.

A D626N mutation in the Cypher/ZASP encoding the Z-disc associated protein was found in all affected individuals in a family and was associated with late-onset DCM [166]. This mutation also proved interesting in that it alters the binding function of the Cypher/ZASP LIM domain and increased its interaction with protein kinase C (PKC), suggesting an association between DCM and the inherited abnormality involved in signal transduction.

Mutations in other signal transduction proteins appear to play a role in late-onset DCM. For instance, in a family with deletion of arginine 14 in the PLN gene, members did not present with DCM until their seventh decade with mildly symptomatic HF [155]. While this finding suggests that PLN mutations should be considered a contributory factor in the development of late onset cardiomyopathy, the finding that other heterozygous individuals with the identical PLN Arg 14 mutation have been reported to exhibit more severe disease at an earlier age (i.e., left ventricular dilation, contractile dysfunction, and episodic ventricular dysrhythmias, with overt HF by middle-age) suggests that other modulatory factors are likely involved in the expression of the DCM phenotype [167].

Another connection between genes involved in DCM and aging phenotypes has emerged with the characterization of LMNA mutations affecting lamin A/C. In addition to a variety of laminopathies including DCM along with neuropathy, lipodystrophy, limb girdle muscular dystrophy (LGMD), and autosomal dominant Emery–Dreifuss muscular dystrophy (EDMD), mutations in LMNA can result in premature aging syndromes or progeria. LMNA mutations have been reported in segmental progerias: Hutchinson–Gilford Progeria Syndrome (HGPS) [168], and Atypical Werner Syndrome [169]. In these aging diseases, the age of onset is quite different. In sharp contrast to Werner Syndrome, which becomes apparent at or shortly after puberty and early adulthood, HGPS manifests early in childhood. Growth retardation can be observed by 3–6 months of age, with degenerative changes in cutaneous, musculoskeletal, and cardiovascular systems showing shortly thereafter; baldness occurs by age 2 and median age of death is 13.5 years with mortality primarily attributable to myocardial infarction or severe HF. While many of the diseases secondary to LMNA mutations arise from dominant missense mutations (e.g., DCM type 1A, LGMD) some are autosomal recessive

(e.g., Charcot–Marie–Tooth disease) and others sporadic (e.g., HGPS, EDMD). It is clear, although largely unexplained, that different mutations in the same gene can lead to diverse dysfunctions and limited phenotypic overlap, with specific tissues targeted in each pathology [170]. An important unanswered question is why defects in nuclear envelope proteins that are found in most adult cell types should give rise to pathologies associated predominantly with skeletal and cardiac muscle and adipocytes. Among three different LMNA-mediated myopathies (i.e., EDMD, DCM and LGMD), cardiomyopathy occurs with the underlying potential of sudden death because of cardiac dysrhythmia. Moreover, the cardiac disease of mutated LMNA is often defined by conduction system and rhythm disturbances occurring early in the course of the disease, followed by DCM and HF. Affected individuals of a family comprised of members heterozygous for the same single nucleotide deletion in exon 6 of the LMNA gene showed different presentations, one with LGMD, one with EDMD, and another with DCM. The intrafamilial variability and mutational pleiotropy observed in this and other studies suggests that other modifying factors (genetic, environmental or epigenetic) likely influence phenotypic expression [171].

While over 20 LMNA mutations (primarily missense defects localized in exons 1 and 3) have been reported to lead to autosomal dominant DCM (type 1A), evidence has been presented that different LMNA mutations can have significant different age-expression. Molecular analysis of two 4-generation white families with autosomal dominant familial DCM and conduction system disease revealed novel mutations in the rod segment of LMNA [172]. A missense mutation (nucleotide G607A, amino acid E203K) was identified in 14 adult subjects of family A with a cardiac phenotype primarily manifested as progressive conduction disease, occurring in the fourth and fifth decades with ensuing death due to HF. In contrast, a nonsense mutation (nucleotide C673T, amino acid R225X) was identified in ten adult subjects of family B with progressive conduction disease occurring with an earlier onset (third and fourth decades), accompanied by ventricular dysrhythmias, left ventricular enlargement, and systolic dysfunction and death caused by HF or sudden cardiac death.

Given the multiplicity of lamin A/C intracellular functions, a clear picture of the pathogenic mechanism by which LMNA mutations causes DCM (and conduction defects) is not yet evident [170–173]. One attractive hypothesis suggests that defective lamin A/C undermines the structural integrity of the nuclear envelope, promoting a mechanical nuclear fragility, and by its interactions with the cytoskeletal desmin results in a whole cell-mechanical vulnerability particularly notable under conditions of constant mechanical stress typical of cardiac and skeletal muscle cells, with resultant impairment of force transmission and contractile function. Other potential pathogenic mechanisms include loss or rearrangement of other LMNA-associated protein (e.g., emerin) and nuclear pore modifica-

tion, changes in heterochromatin relative to the nuclear lamina, and altered gene expression due to disrupted interaction with RNA polymerases and transcription factors (this is further discussed in Chap. 6).

## Restrictive Cardiomyopathy

Restrictive cardiomyopathy (RCM), the rarest form of cardiomyopathy, involves impaired ventricular filling and reduced diastolic volume in the presence of normal systolic function, and normal or near normal myocardial thickness. RCM is most frequently caused by pathological conditions that stiffen the myocardium by promoting infiltration or fibrosis, including endomyocardial disease, amyloidosis, sarcoidosis, scleroderma, storage diseases (e.g., hemochromatosis, Gaucher's disease, Fabry disease, glycogen storage disease), metastatic malignancy, anthracycline toxicity, or radiation damage. Several of the infiltrative diseases resulting in RCM can be inherited, including familial amyloidosis, hemochromatosis, Gaucher's disease, and glycogen storage disease. Most of the congestive HF in the elderly is due to diastolic dysfunction with preserved systolic function suggesting that RCM is an important entity [174]. Amyloidosis is the most prevalent underlying cause of RCM [175], and results from replacement of normal myocardial contractile elements by infiltration and interstitial deposits of amyloid, leading to alterations in cellular metabolism,  $Ca^{2+}$  transport, receptor regulation, and cellular edema. Amyloid myocardium becomes firm, rubbery, and noncompliant, and can also involve the cardiac conduction system presenting with different types of conduction defects and dysrhythmias.

Familial amyloidosis, or hereditary amyloidosis, while overall less common than immunoglobulin amyloidosis (AL), is more frequently associated with RCM, and is most often caused by an autosomal-dominant mutation in the serum protein transthyretin encoded by the *TTR* gene. This gene encodes a protein containing 127-amino acid residues of four identical, noncovalently linked subunits that dimerize in the plasma protein complex. Over 60 distinct amino acid substitutions distributed throughout the *TTR* sequence have been correlated with increased amyloidogenicity of *TTR* [175]. The pattern of myocardial involvement varies according to the specific mutation and has distinct age-expression. A large number of these mutations have been associated with late-onset amyloid cardiomyopathy, as well as with polyneuropathy.

Patients with the Met 30 transthyretin variant, the most prevalent *TTR* mutation, primarily display conduction defects and often require pacemaker implantation [176]. In early-onset cases (i.e., patients younger than 50 years old), cardiac amyloid deposition was most prominent in the atrium and subendocardium but became evident throughout the myocar-

dium in late-onset cases. The Tyr77 mutation, the second most prevalent *TTR* mutation was studied in a large family with 12 affected individuals over four generations; the clinical phenotype is characterized by (sometimes prolonged) carpal tunnel syndrome, beginning between the sixth and seventh decade, with subsequent RCM [177]. Moreover, different ethnic groups have been shown to have varying degrees of susceptibility to cardiac amyloid deposition, while other groups do not have cardiac involvement. Substitution of isoleucine for valine at position 122 of the *TTR* gene has been reported to be more prevalent in African-Americans (estimated to be present in approximately 4% of the black population) [178], and is also associated with the occurrence of late-onset RCM [179]. Molecular analysis revealed that the substitution of isoleucine for valine shifts the equilibrium toward monomer (indicating lower tetramer stability), and favors tetramer dissociation required for amyloid fibril formation and resulting in accelerated amyloidosis [180].

RCM can also be associated with an iron-overload cardiomyopathy that manifests systolic or diastolic dysfunction primarily attributable to increased cardiac iron deposition, and occurs with common genetic disorders such as hemochromatosis [181]. While the precise mechanism of iron-induced HF is not clear, the toxicity of iron in biological systems has largely been attributed to its ability to catalyze ROS generation. Hereditary hemochromatosis is a common autosomal recessive disorder among Caucasians with the genotype at risk accounting for 1:200–400 individuals of Northern European ancestry. Clinical complications appear late in life and often include cardiomyopathy (primarily but not necessarily restrictive) with subsequent development of congestive HF limited to homozygotes. As with many cardiomyopathies, phenotypic expression of the disease shows intrafamilial variability, likely as a result of the effects of modifier genes or to environmental factors. Hereditary hemochromatosis has been linked to pathogenic mutations in the gene coding for HFE, an atypical HLA class I molecule on chromosome 6 (6p21.3), hemojuvelin (HJV or HFE2) on chromosome 1 (most often associated with the juvenile form of hemochromatosis) and more rarely the gene coding for hepcidin (HAMP) on chromosome 19, and the gene encoding serum transferrin receptor 2 [181–183]. Two missense mutations in HFE have been found to be responsible for the majority of cases, C282Y and H63D; the C282Y mutation has a higher penetrance than the H63D mutation, and appears to result in a greater loss of HFE protein function [181]. Iron-overload in the heart resulting from HFE knockout in mice can also lead to increased susceptibility to myocardial ischemia/reperfusion injury as indicated by increased postischemic ventricular dysfunction, increased myocardial infarct size and myocyte apoptosis, with the degree of injury significantly elevated by high-iron diet [184]. While HFE mutations have been reported to be involved in several age-related chronic diseases such as Alzheimer's disease and CAD,

one study suggested that in some populations the same HFE mutations associated with cardiomyopathy and CVDs paradoxically have also shown an increased prevalence (in the heterozygous state) in centenarians (primarily women), suggesting a beneficial role with respect to longevity [185]. This may be because individuals heterozygous for the C282Y mutation tend to have slightly but significantly higher values for serum iron and transferrin saturation and are therefore less likely to exhibit anemia secondary to iron deficiency; however, other studies have failed to replicate this relationship in different ethnic groups [186, 187].

## Conclusion

The increased use of gene profiling in hearts from subjects with age-associated diseases such as cardiomyopathy and HF has begun to define a molecular signature of cardiac dysfunction whose component elements can be informatively compared between diseases, various populations (e.g., ethnic/racial, gender), a variety of treatment regimens (e.g., LV assist devices, pharmacological treatments) and of course, age. Efforts are also being undertaken to define a proteomic profile of age-associated cardiac disease, albeit as we have previously noted, for numerous pragmatic reasons most studies have chosen to target and define limited proteomes (e.g., mitochondrial/organelle-specific, specific classes of protein-modification).

While a number of polymorphic gene variants of candidate-genes in association with age-mediated cardiac diseases have been identified, in general these findings have been extraordinarily difficult to replicate and there are indications that modifying genes and/or environmental and epigenetic factors markedly influence the effects of these genes on the expression of cardiac disease and cardiac phenotype. Newer techniques of gene mapping, including very powerful haplotype mapping, may be applied in defining the genes involved in susceptibility and progression of these diseases in the elderly. Similarly, new techniques are urgently needed and will undoubtedly be developed to elucidate gene–environmental interactions.

In the dawning era of genomic- and post-genomic medicine, although there has not been widespread practical use of genomic information in everyday practice, there are many examples of how this information is beginning to transform the way we look at disease states in terms of diagnosis, prognosis and treatment. The gathered experience with molecular analysis of other non-cardiac diseases will be helpful in developing information to be applied to the management of HF including diagnosis, prognosis, and treatment response. We concur with others [188] that this information may not only be clinically useful but also helpful in advancing research and discovery of new drugs and translational medicine. Therefore, new genomic technologies and information

should enhance our understanding of HF and cardiomyopathies, and in particular the cardiomyopathy of aging.

Although many pharmacodynamic studies have focused primarily on healthy older people, the pathophysiology of CVDs, including HF in the elderly is different than in younger individuals, and this may change the pharmacodynamic response and therapeutic outcome. In spite of the fact that most of the clinical trials on HF have recruited younger men (younger than 65 years old) with systolic dysfunction secondary to ischemic heart disease, in clinical practice, HF is often a syndrome of older women with diastolic dysfunction, perhaps secondary to systemic hypertension. This difference in the pathophysiology of the disease in aging may explain why the survival benefits seen with angiotensin-converting enzyme inhibitors and  $\beta$ -blockers in younger adult are reduced in older people, particularly older women [189, 190]. Finally, the primary goal of the pharmacogenomics of HF, should be to increasingly effectuate a personalized medicine defining the most effective treatment plan (e.g., drug regimens and dosage) to treat disease in patients of specific genetic backgrounds, and ages. Lately, great progress is being made in that direction.

## Summary

- During aging, a significant loss of cardiac myocytes occurs, probably related to programmed cell death (apoptosis). The cumulative effect of this loss may result in significant physiological decline.
- Loss in cardiomyocytes may be secondary to mitochondrial dysfunction, likely caused by chronic exposure to oxidative free radicals, damage to mtDNA (mutations and deletions) and mitochondrial membranes.
- Besides cells loss, other mechanisms involved in cardiac aging are: ROS and oxidative stress, inflammatory mechanisms/signaling, adrenergic, muscarinics, and other cardiac G-protein-coupled receptors, SERCA, thyroid hormone, growth hormone and IGF-1, telomeres and telomere-related proteins, autophagy and cardiac aging, gender, genetic make-up, susceptibility genes, and epigenetic/environmental factors.
- Numerous genes mutations have been identified as a common etiological factor in the more prevalent varieties of cardiomyopathy, HCM and DCM, and also in the more rarely found phenotypes such as RCM.
- Numerous genetic defects have also been implicated in the pathogenesis of metabolic cardiomyopathies (often associated with extra-cardiac presentations) including mitochondrial cardiomyopathies and the cardiomyopathy associated with diabetes.
- Defects in genes encoding sarcomeric and cytoskeletal proteins have been linked to the progression of HCM, a number of which display age-mediated expression.



- Defects in non-sarcomeric/non-cytoskeletal genes have also been associated with familial and sporadic HCM includes defects in metabolic regulatory genes (PRAKG2), signaling proteins (caveolin) and nuclear membrane proteins (Lamin A/C).
- Defects in genes encompassing a wide range of functions have been identified in association with dilated cardiomyopathy (DCM); a number of the associated defects show increased expression in the elderly.
- Research in transgenic animals (primarily mice) models as well as rat and hamster have resulted in informative models of cardiomyopathy and HF which recapitulate clinical phenotypes. Both loss-of-function and gain-of-function models have been used to examine the role of defective metabolic, intracellular signaling and cardiomyocyte contractile and structural components and pathways that can lead to cardiac dysfunction, several with specific aging-related phenotypic expression.
- In addition to providing information concerning pathogenesis, these animal models have often proved useful for testing new treatments of HF.
- Although, most of the clinical trials of HF have recruited younger men (younger than 65 years) with systolic dysfunction secondary to ischemic heart disease, in clinical practice HF is often a syndrome of older women with diastolic dysfunction, perhaps secondary to systemic hypertension.
- The increased use of gene profiling in hearts from subjects with age-associated diseases such as cardiomyopathy and HF has begun to define a molecular signature of cardiac dysfunction whose component elements can be informatively compared between diseases, various populations and of course, age.
- Better understanding of the causes of aging-related diseases including HF, is essential before we can even think if the abolition of human senescence is possible.

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