Article type : Mini-Review

Ageing and longevity genes in cardiovascular diseases

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Running title: ageing and longevity genes in CV function.

Keywords: ageing, cardiovascular diseases, cardiovascular ageing, oxidative stress, inflammation.

Abstract

Over the last century, Western societies experienced a demographic shift driven by increased lifespan and decreased fertility, resulting in the subversion of the world's demographic pyramid. In ageing societies, cardiovascular diseases are the major cause of morbidity and mortality, thus representing a major societal and economic burden. Indeed, ageing associates with the deterioration of a genetic network implicated in senescence and longevity, orchestrating deleterious cellular processes that converge in the structural and functional decline of both the myocardium and the vasculature. In this review, we revise a compendium of genes involved in these processes and delineate possible strategies to interfere with them. Dietary interventions (e.g. intermittent fasting) and sirtuin-activating compounds are among the most promising interventions shown to promote protective effects on the ageing cardiovascular system. We conclude that ageing and longevity genes modulate cardiovascular function by acting on deleterious downstream processes such as inflammation and oxidative stress, thus representing promising targets for the prevention and treatment of age-related cardiovascular dysfunction.

Introduction

Advances of contemporary medicine critically contributed to extend life expectancy; as a result worldwide population is ageing at a unprecedented pace [1]. People over 65 years of age (currently the elderly definition) will be expected to represent one quarter of total population by 2050. Of interest, the oldest segments of the 65-plus population have dramatically grown and centenarians have more than doubled from 2.9 every 10,000 over-65 individuals in 1990 to currently 7.4/10,000. Also, projections foresee a further increment to 23.6/10,000 by 2050 [2]. Despite being extremely positive from an individual perspective, these demographic data raise major concerns for social and health care systems. Indeed, ageing associates with a progressive decline in physiological function of several organs and in line with this, the elderly represent a high-risk population burdened with high morbidity and disability [3].

Ageing is the most important determinant of lifespan, and in rodents ageing *per se* is associated with a decline in cardiac and vascular function even without risk factors suggesting that a genetically determined biological clock governs lifespan and does so by promoting adverse changes causing organ dysfunction [4]. Yet, few insights are available with respect to the molecular pathways determining age-dependent cardiovascular (CV) and cerebrovascular (CBV) diseases and this is also reflected by the lack of direct effective therapies. Ageing, vascular dysfunction and age-dependent CV and CBV disease are mediated by similar molecular mechanisms which predominantly involve production of free radicals and surge of inflammation [5]. Indeed, several animal and human studies showed an association between ischaemic stroke, myocardial infarction and increased systemic and local production of reactive oxygen species (ROS) and inflammatory mediators [6,7]. Of interest, ageing associates with a chronic dysfunctional activation of the inflammatory system occurring in the absence of appropriate infective signals known as inflamm-ageing and thought to be involved in different chronic age-related afflictions [8]. Different genes regulating life span and modulating ageing (i.e. ageing and longevity genes) are major regulator of oxidative stress and inflammation. Accordingly, some of these genes are critically involved in the development of age-dependent CV and CBV diseases as well as being effective molecular targets for their treatment [9] (Figure 1). This review article builds on previously published articles from our research group to delineate a compendium of promising genes implicated in ageing and age-related CV diseases. Moreover, it discusses their potential as targets for future therapeutic interventions.

Ageing genes

The Adaptor Protein p66^{Shc}: The adaptor protein p66^{Shc} is considered to play a key role in driving cellular senescence by regulating mitochondrial reactive oxygen species (ROS) formation and thus, cellular apoptosis [10]. Along with p46^{Shc} and p52^{Shc}, p66^{Shc} is encoded by the human ShcA locus with major roles in different cellular signalling pathways [11]. Mechanistically, p66^{Shc} translocates into the mitochondrion upon protein kinase C-β-mediated Ser36 phosphorylation. Mitochondrial p66^{Shc} initiates the oxidation of cytochrome c, thus resulting in enhanced hydrogen peroxide production and increased oxidative stress, eventually leading to the activation of pro-apoptotic pathways [10,12,13]. With ageing, vascular ROS production increases, thus reducing protective nitric oxide (NO) bioavailability and leading to endothelial dysfunction and CV disease [14,15]. In accordance with its master role in regulating ROS production, tissue levels of p66^{Shc} are increased in vessels from elderly patients as compared to young individuals [16]. Hence, p66^{Shc} gained increasing attention as a promising molecular target to reduce the burden of age and age-related CV diseases [10]. Indeed, mice lacking *p66^{Shc}* show an increased lifespan of up to 30% which goes along with decreased levels of intracellular ROS and cellular resistance to paraquat-induced oxidative stress [17]. Notably, *p66$^{Shc-/-}$* mice also exhibit preserved endothelial NO bioavailability and stable aortic superoxide levels during the course of ageing, suggesting that these animals are protected from age-dependent endothelial dysfunction mediated by oxidative stress [18]. In line with the recognized role of dysfunctional endothelium as *primum movens* of atherosclerosis [19], the expression and activity of p66^{Shc} positively correlates with different CV risk factors (i.e. hyperglycaemia, hyperlipidaemia, and hyperhomocysteinaemia) in humans [20–22]. The diameter of the retinal microvasculature has been proposed to serve as a marker of vascular health and, being associated with CV risk. Recently, wider retinal arterioles and narrower venules – indicators of good vascular health – have been reported to associate with blunted *p66^{Shc}* mononuclear cell expression and lower levels of oxidative stress markers in healthy individuals being physically active as compared to sedentary subjects (with or without CV risk factors). Of note, these differences have been linked to hypomethylation of the *p66^{Shc}* promoter suggesting once more epigenetics as an important modulator of vascular ageing [21]. In accordance with its association with CV risk factors, p66^{Shc} has been shown to be implicated in atherosclerotic plaque formation. Napoli *et al.* [22] found that high-fat fed mice lacking p66^{Shc} are less susceptible to develop early atherosclerotic lesions while exhibiting reduced levels of systemic oxidative stress.

The detrimental role of p66^{Shc} was further investigated using male mice deficient for ApoE and p66^{Shc}, revealing that p66^{Shc}-deprivation attenuates atherosclerotic plaque formation also in advanced stages [23]. As a further step, the putative role of p66^{Shc} has been investigated in the tremendous clinical sequelae of advanced atherosclerotic lesions, such as myocardial infarction and ischemic stroke. Of interest, monocytes of patients presenting with acute coronary syndrome (i.e. either ST-elevation or non-ST-elevation myocardial infarction) showed augmented *p66^{Shc}* transcript levels, suggesting a potential role of this protein in myocardial ischaemia [24]. Despite an early preclinical study employing a non-recirculating Langendorff approach suggested *p66$^{Shc-/-}$* hearts to be less susceptible to ischemia-reperfusion (I/R) injury [25], transient *in vivo* ligation of the left anterior coronary artery in *p66$^{Shc-/-}$* mice resulted in increased myocardial injury due to blunted activation of protective pathways (i.e. Akt and Stat 3) thus increasing mitochondrial swelling and cellular apoptosis [26]. In the brain, on the other hand, mounting evidence points towards a detrimental role of p66^{Shc} in the setting of I/R injury. Of interest, *p66$^{Shc-/-}$* mice subjected to transient middle cerebral artery occlusion exhibit reduced stroke size and blunted neurological dysfunction after stroke, which is paralleled by attenuated ROS production locally and systemically [27]. Intriguingly, these effects are also present when small interfering RNA targeting p66^{Shc} is injected intravenously after the ischaemic event upon reperfusion, an approach with high translational value. In line with the above, peripheral blood monocytes obtained from patients with ischaemic stroke display transiently increased *p66^{Shc}* mRNA levels which correlate with short-term neurological outcome if thrombolytic treatment is initiated [28].

Mitochondrial ROS formation is actively involved in hypertension-induced vascular dysfunction where cyclic stretch stress disrupts the cellular redox balance and impairs the endothelial homeostasis [29]. Accordingly, human aortic endothelial cells exposed to pathologic cyclic stress exhibit enhanced p66^{Shc} phosphorylation, ROS production and blunted NO bioavailability [30]. Similarly, aortic endothelial cells isolated from spontaneously hypertensive rats show augmented Ser36 phosphorylation of p66^{Shc}, which associates with enhanced superoxide production in aortic homogenates [30]. At the cardiac level, long-standing hypertension-related pressure overload causes left-ventricular dysfunction and myocardial remodelling, leading to dilated cardiomyopathy and heart failure. Notably, recent evidence supports the notion that p66^{Shc} may represent an important nexus that orchestrates oxidative stress and cardiac dysfunction in a murine model of pressure overload-induced heart failure [31]. In patients with CV risk factors, Angiotensin-II

(AngII) is considered to be a critical mediator promoting cardiac remodelling and ageing. Notably, mice lacking $p66^{Shc}$ are protected against cardiac AngII-induced hypertrophic remodelling via reduced apoptotis [32].

Taken together, these data suggest that $p66^{Shc}$ is an important player implicated in the regulation of CV (dys-)function in ageing and highlight its potential as a therapeutic target to reduce the burden of age-related CV diseases. Yet, given the different effects of p66Shc modulations in different organs, tissue selective inhibition would be required. While its short-term activation might protect the infarcted myocardium, $p66^{Shc}$ inhibition may reduce cerebral ischaemic injury and prevent endothelial dysfunction, atherosclerosis, and diabetic vascular disease. This complexity rose safety concern on the potential use of p66Shc modulators in the clinical setting and to date no approved therapeutic targets this gene.

Mammalian target of rapamycin: The mammalian target of rapamycin (mTOR) is a highly conserved serine/threonine kinase embedded in a sophisticated network governing cellular growth and metabolism upon nutrient-sensing and hormonal stimuli [33]. mTOR exerts its effects by forming two structurally and functionally distinct multiprotein complexes, namely mTORC1 and mTORC2, thus regulating different downstream processes [34]. These protein complexes emerged as critical players of CV integrity during embryonic development as well as in adulthood [34,35]. Indeed, while adult mice lacking cardiac-specific mTORC1 activity develop fatal dilated cardiomyopathy at baseline, these animals display impaired hypertrophic response and accelerated heart failure progression upon pressure-induced overload stress [36]. Similarly, deprivation of mTORC2 in the murine heart via *RPTOR Independent Companion Of MTOR Complex 2* (*Rictor)* deletion causes cardiomyocyte death and promotes left-ventricular dilation and fibrosis, resulting in profound cardiac dysfunction [37]. On the opposite, partial genetic or pharmacological inhibition (through rapamycin) of mTORC1 has consistently been linked to longevity, and confers protection against multiple age-related CV diseases by modulating various downstream pathways, including autophagy, oxidative stress and inflammation [36,38–40]. While mTORC2-activity is less sensitive to rapamycin treatment and requires long-term administration to be effectively blocked, mTORC1 activity was shown to be immediately inhibited following acute application [40]. Traditionally being used as an immunosuppressant, beneficial effects of rapamycin on CV function were first described in renal allograft patients withdrawn from cyclosporine, in which add-on treatment with rapamycin resulted in lower blood pressure levels despite the use of less

antihypertensive drugs [41]. Likewise, age-related mTORC1 activation in mice is thought to be involved in the development of endothelial dysfunction via increased ROS formation and blunted NO bioavailability, effects partially reversed by rapamycin treatment [15,42,43]. With age, arterial walls lose elastic tissue in favour of collagen deposition, a process termed arterial stiffening, which is characterized by increased pulse wave velocity, representing a strong predictor of CV events [44]. Notably, Lesniewski *et al.* observed attenuated arterial stiffening in mice upon treatment with dietary rapamycin via decreased vascular collagen content, while content of elastin did not differ between groups [43]. In line with this notion, kidney transplant recipients whose therapy regimen is switched to rapamycin display improved carotid-femoral pulse-wave velocity, proposing mTOR-inhibition as a potential therapeutic strategy against arterial stiffening in selected patients [45]. Besides, Flynn *et al.* reported lately that mTOR-inhibition through rapamycin exerts beneficial effects on cardiac function and is capable to prevent age-related hypertrophic remodelling under unstressed conditions, even if administered late in life, suggesting a protective role independent of arterial stiffening [39]. In this context, low-grade sterile inflammation (i.e. "*inflamm-ageing"*) is a hallmark of ageing deeply linked to various age-related CV diseases, including atherosclerosis [46,47]. Very recently, the endothelial-specific deletion of the endogenous mTORC-1 inhibitor PRAS40 was reported to promote atherogenesis in atherosclerosis-prone animals via induction of pro-inflammatory signalling [48]. Despite several lines of evidence proposing an important role for mTORC1 in the modulation of inflammation, in a recent study rapamycin administration failed to ameliorate inflamm-ageing markers in a mouse model of accelerated ageing despite confirming its protective role on frailty and health span in general [49]. Yet, mTORC1 modulation through rapamycin or derivatives has been proven effective in reducing experimental atherosclerosis, and these drugs are currently applied locally in daily clinical practice to reduce arterial restenosis after stenting [49,50].

Albeit a bulk of evidence suggests a beneficial role of partial mTOR inhibition in maintaining CV health and integrity during the course of ageing, studies assessing selective blockade of mTORC signalling or its downstream targets (e.g. S6K1) are scarce. Thus, future studies are warranted to investigate the specific modulation of mTORC-1 and mTORC2-dependent pathways using genetic or pharmacological approaches.

Longevity genes

Sirtuins: The sirtuin family comprises seven proteins (SIRT1 through 7) governing nicotinamide adenine dinucleotide (NAD^+)-dependent deacylase reactions (such as deacetylation, demalonylation, desuccinylation) and, therefore represent important players regulating key aspects of ageing [51]. In mammals, various genes acting in nutrient-sensing pathways, including *FOXO3* (for details see below) and *sirtuins*, have been identified as crucial regulators of CV senescence, and variants within these genes have been linked to exceptional longevity across numerous studies [52]. Of interest, most sirtuins have been shown to play important roles in the modulation of oxidative stress, inflammation, autophagy, cellular senescence, and apoptosis, processes deeply involved in the pathophysiology of CV diseases [53]. As vascular levels of NAD^+ and sirtuin activity steadily decline during the course of ageing [54,55], unravelling their role in age-related CV afflictions is of particular interest. In *ApoE*$^{-/-}$ mice fed on a high-fat diet, endothelial cell-specific Sirt1 overexpression as well as the administration of its pharmacological activator SRT3025 dampens atherosclerosis [56,57]. Oppositely, Sirt1 inhibition associates with increased plaque formation in atherosclerosis-prone experimental models [56,57]. Notably, *ApoE*$^{-/-}$ mice display increased endothelial superoxide formation and enhanced NF-kB-dependent expression of adhesion molecules upon *Sirt1* deprivation, suggesting that *Sirt1* may protect from atherosclerosis by blunting inflammation and oxidative stress [58]. Similarly, while mice lacking *Sirt1* specifically in cardiomyocytes display exacerbated myocardial injury upon I/R injury, its cardiac overexpression associates with reduced infarct size via blunted apoptosis [59]. Likewise, *in vitro* studies found that the upregulation of the mitochondrial sirtuins (*Sirt3*, *4* and *5*, respectively) maintain cardiomyocyte structure and function in the setting of I/R, proposing a protective role of these proteins during I/R in the heart [60–62]. For example, *Sirt3* deficient animals show increased cardiac damage [63], profound coronary microvascular dysfunction as well as impaired remodelling upon myocardial ischaemia [64]. Additionally, sirtuins have been hypothesized to be implicated in the pathophysiology of cerebral I/R injury. Recently, we showed that mice lacking endothelial-specific *Sirt6* display increased infarct volumes, mortality and brain extravasation of large molecules following transient middle cerebral artery occlusion, indicating that SIRT6 plays a protective role in ischaemic stroke by preserving blood-brain barrier integrity [65]. We further showed that post-ischaemic SIRT6 overexpression reduces stroke size by 50% and ameliorates neurologic outcome, highlighting its potential as a therapeutic target in this context [65]. Confirmatory *in vitro* experiments employing primary human cerebral microvascular endothelial cells increased the translational relevance of our findings, which has been further strengthened by

the assessment of *Sirt6* expression in peripheral blood mononuclear cells (PBMCs) obtained from stroke patients. Of interest, we found that its expression in PBMCs closely correlates with stroke outcome [65]. On the contrary, we previously studied the detrimental role of *Sirt5* in the context of ischaemic stroke [66]. Specifically, our findings suggest that PBMCs and cerebrovascular tissue obtained from stroke patients and mice following experimental I/R brain injury, respectively, display enhanced Sirt5 gene expression. Likewise, SIRT5 gene deletion or silencing using small-interfering RNA resulted in reduced infarct size, post-stroke neuromotor deficit and BBB permeability through blunted occludin degradation, effects reproduced *in vitro* in human brain microvascular endothelial cells exposed to hypoxia/reoxygenation [66].

Increased fibrosis, left-ventricular hypertrophy and dilation are hallmarks of cardiac ageing and key causes of heart failure. Notably, SIRT3 levels steadily decline during hypertrophic remodelling irrespective of the model used [67,68]. Accordingly, ageing mice deficient for cardiac-specific *Sirt3* develop cardiac hypertrophy and fibrosis, while its overexpression protects from hypertrophic stimuli, at least in part, by enhanced FOXO3a activation [69]. A bulk of evidence suggests that the effects mediated by SIRT1 and SIRT3 may be also beneficial in the setting of hypertension, a potent risk factor for numerous CV diseases. Indeed, in mice with Angiotensin-II-induced hypertension, *Sirt1* overexpression alleviates vascular remodelling, abrogates ROS formation and exerts antihypertensive effects [70]. Furthermore, mice lacking *Sirt3* develop spontaneous pulmonary arterial hypertension (PAH) which associates with aberrant mitochondrial function. Interestingly, in rats with monocrotaline-induced PAH, disease phenotype can be reversed upon the rescue of *Sirt3* deficiency using adenovirus gene therapy, implying a causative role of SIRT3 in PAH. In line with this notion, a loss-of-function SIRT3 polymorphism associates with PAH in humans, which underscores the protective role of this protein in PAH [71]. On the contrary, the role of Sirt5 in the setting of cardiac hypertrophy remains to be fully characterized since opposite findings have been reported so far by using genetically deficient mice undergoing transverse aortic constriction [72,73].

In summary, with a few exceptions these findings point towards a protective role of most sirtuins in atherosclerosis, cardiac I/R injury, cardiac remodelling, hypertension and stroke. Few exceptions need to be considered with respect to specific sirtuins, their targets, route of administration (i.e. systemic vs local) or dosage. These effects may, at least in part, be mediated

by the activation of different downstream pathways crucially involved in ageing and longevity (such as FOXO3 and $p66^{Shc}$).

Beclin 1: Macroautophagy (hereafter referred to as autophagy) is a cytoprotective process that maintains cardiac structure and function during the course of ageing [74]. Physiological (e.g. caloric restriction), pharmacological (e.g. spermidine) or genetic induction of this process promotes longevity and exerts cardioprotective effects across different species, including mice [75–77]. Accordingly, *Beclin 1* knock-down has been associated with defective autophagy and shortened lifespan [77]. Conversely, knock-in mice bearing a *Phe121Ala* mutation in *Beclin 1* (*$Becn1^{F121A/F121A}$*) which reduces the interaction with the negative regulator Bcl-2 and results in enhanced autophagy, associates with blunted age-related structural changes of the heart (i.e. cardiac fibrosis) and improved health- and lifespan [78]. Of interest, *Beclin 1* overexpression exerts deleterious effects in mice subjected to thoracic aortic constriction, as evidenced by impaired systolic function and accentuated pathological remodelling, indicating that enhanced autophagy may be detrimental in the setting of pressure-related cardiac remodelling [79]. Furthermore, Razani *et al.* showed that Beclin 1 protein levels are not significantly altered in aortic lysates obtained from atherosclerosis-prone *$ApoE^{-/-}$* mice, indicating that atherosclerotic plaque formation may not depend on upstream components of the autophagic pathway. Notably, the complete loss of macrophage autophagy, however, promotes compromised cholesterol efflux capacity and enhanced foam cell development, resulting in overt plaque formation, implying a protective role of basal autophagy in atherogenesis [80]. Hence, Beclin 1, among other proteins regulating autophagy (e.g. mTORC1), may represent a promising target for cardioprotective interventions during ageing. Yet, the effects of its modulation seem complex and might depend based on the disease and the magnitude of the intervention. More studies are required to investigate the specific role of Beclin 1 in the different age-related CV diseases.

Klotho: So far, two isoforms (i.e. αKlotho and βKlotho) of the Klotho protein have been described, both important components of the fibroblast growth factor receptor complex. The constitutively-occurring ectodomain-shedding of αKlotho liberates a soluble form, which acts as a circulating hormone regulating a plethora of cellular processes including the inhibition of growth factors, such as insulin-like growth factor 1 (IGF1) and transforming growth factor β1 (TGFβ1) [81]. Interestingly, mice bearing a mutation in the *Klotho* gene display ageing-like phenotypes across different tissues with an average life-span of 60.7 days [82]. Conversely, *Klotho*

overexpression increases lifespan by 20-30% and alleviates ageing-like phenotypes [83]. Furthermore, it promotes cardioprotection [84], and blunts oxidative stress [85]. On the other side, *Klotho*$^{+/-}$ mice display impaired aortic eNOS activity and attenuated endothelial SIRT1 expression, resulting in arterial stiffening and hypertension. Interestingly, treatment with a SIRT1 activator (i.e. SRT1720) was able to rescue the deleterious effect of *Klotho*-deprivation on vascular structure and function [86]. In line with the fact that mice partially defective for *Klotho* are more prone to develop atherosclerosis [82], humans bearing a functional variant for *KLOTHO* display an increased risk of early-onset coronary artery disease [87].

Forkhead Transcription Factors: The forkhead transcription factors (FOXOs) represent a subfamily of highly-conserved proteins regulating longevity by modulating insulin/IGF-1 signalling [88]. In humans, the gene encoding the transcription factor FOXO3 has been consistently linked to longevity [89,90]. In Drosophila, modest overexpression of *Foxo* exerts cardioprotective effects and ameliorates the age-related decline in cardiac function [91]. Accordingly, *Foxo3*$^{-/-}$ mice display a more pronounced hypertrophic response upon pressure-induced overload as compared to their control littermates. Likewise, cultured cardiomyocytes expressing *Foxo1* or *Foxo3* show attenuated Angiotensin-II induced hypertrophic growth, suggesting that *Foxo*s regulate cardiomyocyte growth [92]. On the other hand, overexpression of both, *Foxo1* and *Foxo3a* results in reduced eNOS protein expression. In line with this notion, aortic lysates obtained from *FOXO3a*$^{-/-}$ mice have decreased endothelial eNOS protein expression [93]. Yet, FOXO factors positively regulate the expression of key ROS-scavenging enzymes such as MnSOD (manganese superoxide dismutase), catalase, and GADD45 [94,95] and their inactivation associates with increased intracellular ROS and accelerated atherosclerosis [96]. Albeit accumulating evidence points towards a beneficial role of FOXOs within the longevity network, more research is warranted to understand the processes governed by these proteins in the CV system during health and disease.

Ageing and longevity genes as targets to improve CV outcome: future perspectives

The clear pre-clinical evidence pointing at ageing and longevity genes as pivotal mediators of age-dependent CV disease together with the global burden of an ever-ageing society call for great efforts in translating these results into effective clinical strategies. While the "*bench-to-bedside*" translation occurred smoothly for mTOR and its inhibitors, this is not the case for other potential therapeutic targets in which activity modulation in patients is not easy to obtain. Sirtuins represent

a paradigmatic example and major advances have been obtained as different clinical trials evaluating sirtuin boosters showed protective effects in cardiovascular and metabolic diseases. Different approaches can be used to increase sirtuin activity, such as dietary interventions (e.g. fasting, intermittent fasting or ketogenic diet) and administration of NAD^+ boosting or sirtuin-activating compounds (Small-Molecule Allosteric Activators of Sirtuins or STACs) [97–99] (Figure 2).

Beyond its fundamental role in redox balance, NAD^+ is the essential substrate of sirtuin enzymatic activity. Accordingly, NAD^+ was shown to play pivotal roles in cell signalling and increased cellular supplies of this mediator mimic caloric restriction and associate with stress resistance and prolonged lifespan [98]. Also, cellular NAD^+ is significantly down-regulated in ageing while its supplementation associates with several protective effects on metabolism and CV function in different experimental models [55,100]. Besides the supplementation of NAD^+ precursors, such as nicotinamide or nicotinamide mononucleotide (NMN), enzyme induction of the NAD^+ synthesis pathways or blockage of NAD^+ consuming pathways (i.e. inhibition of Poly [ADP-ribose] polymerase (PARP1) via INO-1001) may represent feasible strategies to increase intracellular NAD^+ levels [100–102]. Among STACs, resveratrol and other natural phenols have been long-term described and characterized [103,104]. Despite being a potent activator of SIRT1 and having shown ability to extend lifespan in yeast [105], resveratrol as well as other phenols lacks specificity and can induce the activity of other SIRTs as well as non-sirtuin targets [106,107]. Then, different STACs have been generated with increased activity and specificity all acting as allosteric activator of SIRT1. Both NAD^+ boosters and STACs have proven beneficial actions in different animal models of disease. Treatment with the SIRT1 activator SRT1720 was able to reverse ageing-associated endothelial dysfunction in mice through decreased oxidative stress and vascular inflammation [108]. Similarly, in monkeys, resveratrol treatment blunted arterial stiffening and inflammation. Treatment with NMN and other NAD^+ boosters showed similar beneficial effects on vascular function in rat models of disease as well as increasing angiogenesis ability of the aged vasculature [109]. Increased cardiac NAD^+ levels also showed protective features on I/R myocardial injury as well as being indicated as possible mediators of the beneficial effect of preconditioning [110,111]. Similarly, several studies suggest that NAD^+ boosters may protect against hypertrophy and cardiac remodelling in several experimental models [112,113], while the role of STACs in this setting remain to be fully elucidated with opposite effects of the over activation of different sirtuin at different level being reported [114].

Among STACs, resveratrol and SRT2104 have been investigated in several randomized clinical trials with metabolic or cardiovascular outcome. Although contrasting results have been so far reported by different clinical trials, treatment with resveratrol has shown modest beneficial evidence on metabolic parameters (e.g. insulin sensitivity, body weight and circulating lipids) in obese, diabetic and metabolic syndrome patients, while these effects were not reported in healthy people [115–118]. Of interest, in patients with coronary artery disease, resveratrol could improve endothelial and diastolic function [119]. Only few trials investigated NAD^+ boosters as sirtuin activator in the contest of CV disease. NRPT, NR, NMN and INO-1001 showed good safety profiles [120–122] and promising preliminary results, many trials investigating their effect on heart failure, atherosclerosis and other CV outcome are currently underway [114].

Despite of the impressive amount of evidence, none of the approaches described above have so far translated into an effective strategy to counteract CV ageing and disease. Potentially divergent roles for different SIRTs in different CV conditions and the lack of specificity of the available pharmacological approaches might partly account for this discrepancy. Similarly, other antioxidants have failed in proving efficacy in large clinical trials. However, the lack of benefit of antioxidants in clinical trials does not disprove the central role of oxidative stress in cardiovascular ageing which is rather well-defined and proven by a plethora of experimental and clinical findings. Many explanations have been proposed to address this paradox: (i) the choice of antioxidants tested in current trials was mostly driven by their availability and safety profiles rather than their efficiency as antioxidants (e.g. vitamins); (ii) the trials may not have lasted long enough: oxidative stress acts on the vessels for decades, thus, months or a few years of antioxidant therapy may not be enough for reversing its deleterious effects; (iii) preventing ROS production as mostly done in the experimental setting rather than increase ROS scavenging by using non-specific antioxidants may lead to better results, particularly as ROS may sequester in cell compartments and are rapid in their mode of action. Thus, it might be more promising (i) to test genetic approaches (e.g. RNA interference, antisense technology) to prevent the formation of ROS, (ii) selecting an appropriate population with markedly increased oxidative stress and (iii) with long-term follow-up. Accordingly, understanding the molecular mechanisms regulating the age-dependent modulation of ageing and longevity genes is of utmost importance to generate novel specific and effective therapeutic approaches [5]. Standing at the crossroad between genetic background and environmental factors, epigenetic modifications have emerged as significant modulator of gene expression during the life course with crucial role on vascular and heart function [123]. A careful

description of the epigenetic changes characterizing the elderly might pave the way for novel strategies aiming at reprogramming the genetic maladaptive changes occurring with ageing thus reducing their burden on the CV system.

In recent years, senescence-associated secretory phenotype (SASP), a cellular process characterized by the production of extracellular modulators, such as pro-inflammatory cytokines, aroused increasing attention in ageing research [124]. Interestingly, novel findings indicate that Klotho suppresses the maladaptive activation of the endoplasmic reticulum and Golgi in senescent monocytes, which may, in turn affect the acquisition of SASP [125]. To our knowledge, however, so far no direct link between the genes previously outlined and SASP in the setting of CV diseases has been established, although a complex intersection among different axes, specifically NF-κB-dependent signalling, may exist [126]. The term "senolytics" comprises a broad family of small molecules that selectively promote death of senescent cells and target a variety of subcellular pathways, including FOXO4-dependent signalling, and have been shown to be effective under conditions of accelerated ageing and may therefore represent a feasible strategy to maintain or even restore tissue homeostasis during ageing [127,128]. Albeit preliminary results on humans obtained during a clinical trial using a combination of senolytic drugs show a clear decline in adipose tissue senescent cells in subjects with diabetic kidney disease, large-scale studies are warranted to investigate their efficacy and safety in the setting of CV diseases [129,130]).

Conclusions

By acting on common cellular mechanisms such as oxidative stress and inflammation, ageing and longevity genes hold clear roles in the pathophysiology of different CV diseases. Specifically, extensive preclinical evidence exists for a protective role of most sirtuins and *Klotho*, while $p66^{Shc}$ has emerged as a potential mediator of CV dysfunction. Modulating the expression or the activity of such targets will be vital to prevent and treat age-dependent CV conditions.

Acknowledgement

GGC received support for this work from: the Swiss National Science Foundation [310030_175546], the Swiss Heart Foundation, the Alfred and Annemarie von Sick Grants for Translational and Clinical Research Cardiology and Oncology and the Foundation for Cardiovascular Research–Zurich Heart House. GGC is also the recipient of a Sheikh Khalifa's Foundation Ass. Professorship at the Faculty of Medicine, University of Zurich.

Conflict of interests

None.

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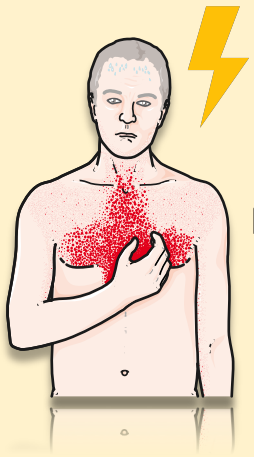
Figure legends

Figure 1. The hallmarks of the ageing cardiovasculature. During the course of ageing, the imbalance of genes involved in ageing (red) and longevity (green) fuels deleterious cellular processes that coincide with the functional and structural decline of the arterial system (left) and the heart (right). Note that the aged myocardium displays ventricular hypertrophy and fibrosis, whereas the ageing vasculature exhibits endothelial dysfunction, arterial stiffening, and atherosclerosis, culminating in increased risk of myocardial infarction, stroke and heart failure. * indicates direct or indirect involvement of the ageing genes mentioned in disease initiation or progression. For details see main text.

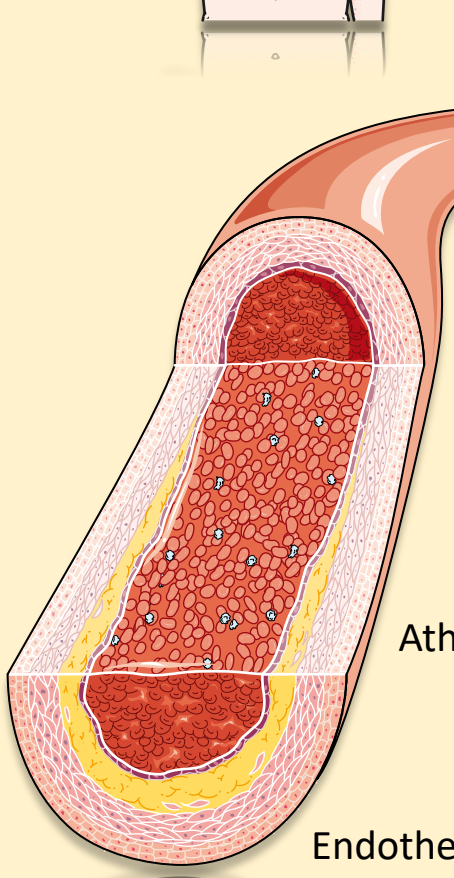
Figure 2. Dysregulation of the outlined signalling network which impacts longevity and ageing converges on the activation of pro-inflammatory pathways and perturbated redox signalling. Dietary interventions (e.g intermittent fasting or caloric restriction) act on downstream targets such as sirtuins (which deacetylate FOXO) and mTOR, resulting in enhanced expression of genes implicated in stress resistance and mitochondrial biogenesis. Likewise, the administration of sirtuin-activating compounds, such as STACs, may represent feasible strategies to combat the burden of age-related cardiovascular maladies. Substrate boosting through NAD+ precursor supplementation, enzyme induction or the inhibition of degrading pathways may be a novel approach to activate downstream targets such as sirtuins which impact many cellular functions, including inflammation and stress resistance. Finally, epigenetic reprogramming of circulating or bone marrow-derived cells followed by autologous transplantation may be beneficial to rescue maladaptive changes of the human genome occurring with ageing.

The Aging Cardiovasculature



Stroke*

Myocardial Infarction*



Arterial Stiffening*

Atherosclerosis*

Endothelial Dysfunction*

mTOR

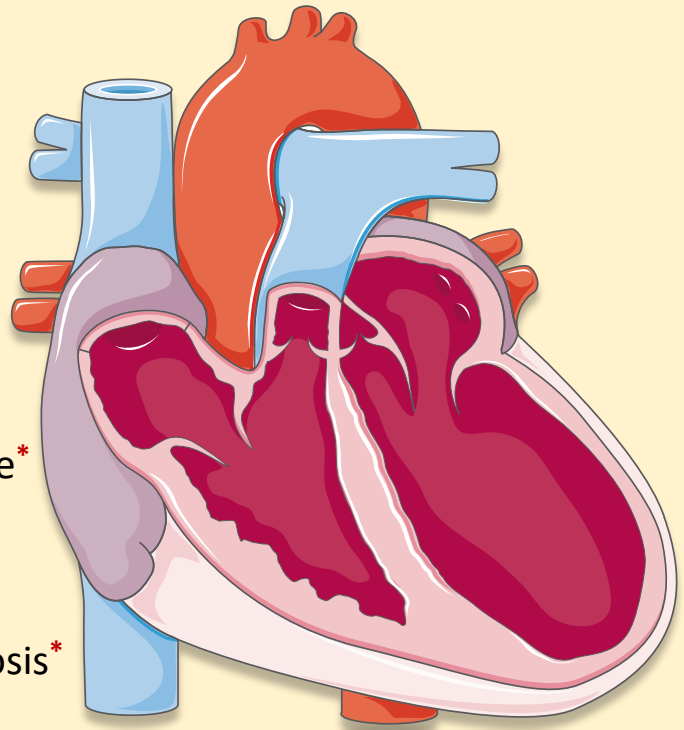
p66^{Shc}

SIRT

BECN 1 *FOXO*

KLOTHO

Hypertension*



Heart Failure*

Fibrosis*

Ventricular Hypertrophy*

[Age]

Longevity Genes

Aging Genes

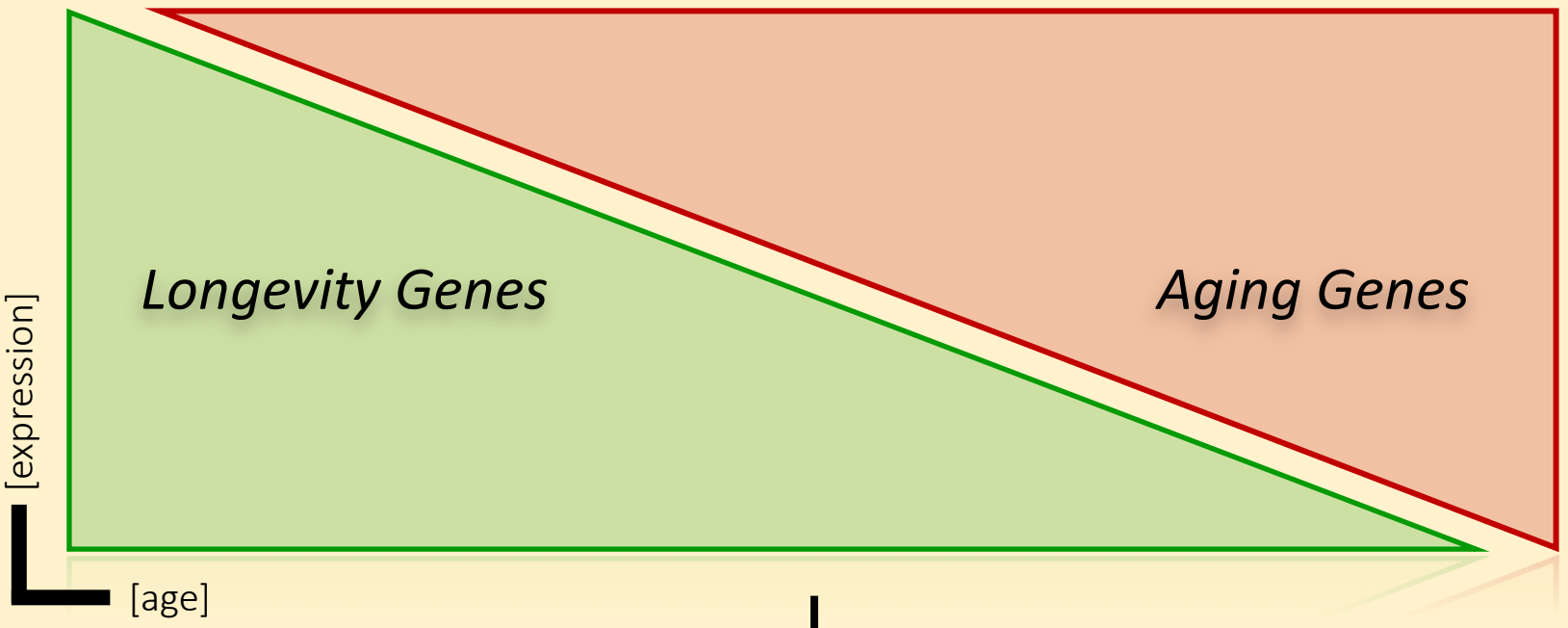
Aging and Longevity Genes

Dietary interventions

Activating or inhibiting compounds

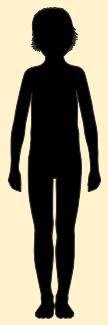
Epigenetic reprogramming

Substrate boosting



Oxidative stress

Inflammation



Age-related cardiovascular dysfunction