Free Radical Biology and Medicine 220 (2024) 262–270

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

The role of sirtuins and uncoupling proteins on vascular aging: The Northern Manhattan Study experience

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A B S T R A C T

Aging affects all organs. Arteries, in particular, are among the most affected. Vascular aging (VA) is defined as age-associated changes in function and structure of vessels. Classical VA phenotypes are carotid intima-media thickness (IMT), carotid plaque (CP), and arterial stiffness (STIFF). Individuals have different predisposition to these VA phenotypes and their associated risk of cardiovascular events. Some develop an early vascular aging (EVA), and others are protected and identified as having supernormal vascular aging (SUPERNOVA). The mechanisms leading to these phenotypes are not well understood. In the Northern Manhattan Study (NOMAS), we found genetic variants in the 7 Sirtuins (SIRT) and 5 Uncoupling Proteins (UCP) to be differently associated with risk to developing VA phenotypes. In this article, we review the results of genetic-epidemiology studies to better understand which of the single nucleotide polymorphisms (SNPs) in SIRT and UCP are responsible for both EVA and SUPERNOVA.

1. EVA and SUPERNOVA

Age is the most important and independent risk factor for all chronic diseases, including vascular disorders (atherosclerosis, stroke, myocardial infarction), vascular cognitive impairment and dementia[1,2]. Like other organs across the age, vessels are damaged by inflammation, oxidated circulating lipids, reactive oxygen species (ROS), mitochondrial dysfunction, and genetic predisposition[3]. The result of these persistent insults and damage leads to vascular aging (VA)[3]. Although the definition is still debated, VA includes all age-associated changes in vessels function and structure, that start by endothelial dysfunction that progress to clinically evident phenotypes, such as intima-media thickness (IMT) [4], carotid plaque (CP), and arterial stiffness (STIFF) [5], that may be considered markers of VA[5].

Individuals have different predisposition to developing atherosclerosis and cardiovascular events and, therefore, to early vascular aging (EVA)[6]. EVA, refers to premature modifications in artery structure and function, mimicking the effects of accelerated aging[3]. Conversely, individuals having low STIFF, no arterial plaques, or less IMT than expected for their age, even in presence of vascular risk factors (RFs), are considered to have supernormal vascular aging (SUPERNOVA)[7]. Therefore, EVA and SUPERNOVA individuals may be defined as those with discrepancy of their chronological age from biological VA.

SUPERNOVA are those with elastic arteries despite exposure to classical RFs[7]. Epidemiological studies reported of subjects that, beside their chronic exposure to RFs, were protected from major cardiovascular complications[8,9]. Data from the Malmö Diet and Cancer Study Cohort demonstrated that SUPERNOVA subjects had VA $\leq$6 years than their chronological age, and had an age- and sex-adjusted rate of cardiovascular events ~40 % lower than individuals with normal VA and EVA, despite a greater chronological age, and a similar burden of RFs[6]. These findings are suggestive as beyond the classical and less classical RFs, there are other mechanisms predisposing or protecting individuals to VA.

Genetics and epigenetics are main human components involved on individual variability to develop chronic diseases. Several findings, belonging from genome-wide association studies (GWAS), candidate genes method, and epigenetic analysis, associated different genetic variants with risk to EVA predisposition[10]. Previously, by using a multi-ethnic cohort population from the Northern Manhattan Study

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https://doi.org/10.1016/j.freeradbiomed.2024.05.010
Received 23 March 2024; Received in revised form 4 May 2024; Accepted 7 May 2024
Available online 8 May 2024

(NOMAS)[11], we demonstrated, as diverse single nuclear polymorphisms (SNPs) of Sirtuins (SIRT), and uncoupling proteins (UCP), associated with different risk to develop phenotypes of atherosclerosis, and therefore VA[12–15]. All mechanisms regulated by SIRT and UCP are significantly implicated in processes leading or protecting to VA [16]. In this article, we review the results of genetic-epidemiology studies to better understand which of the SNPs in SIRT and UCP are responsible for both EVA and SUPERNOVA.

2. Sirtuins and UCPs: General description

2.1. Sirtuins

Sirtuins (SIRT) belong to a conserved family of proteins, originally described in *Saccharomices cerevisiae* as silent mating type information regulation-2 (Sir2), involved in regulating aging and longevity[16]. SIRT1, an homolog of Sir2, was the first described in mammals where then, other six members of this family were subsequently identified[16]. SIRT are defined as class III histone deacetylases that require nicotinamide adenine dinucleotide (NAD^+) as cofactor[16] and, therefore, are considered sensors of cellular energetic and oxidative balance[17].

SIRT regulate several cellular pathways, are distributed in all metabolically active tissues and have different subcellular localization [17–19]. SIRT1, 2, 6 and 7 are mainly located in the nucleus, SIRT1 and 2 are also present in the cytoplasm, while SIRT3, 4 and 5 localize in the mitochondria[16].

SIRT1 deacetylates histone-target, promoting gene transcription [20], and non-histone proteins such as p53, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), and Forkhead box protein O family (FOXO), involved in apoptosis, mitochondrial biogenesis, and oxidative stress regulation[18]. SIRT1 activation protects from neurodegenerative disorders[21], regulates stem cell differentiation[22], blunts replicative senescence in fibroblasts [23], and is involved in several protective mechanisms associated with longevity and metabolism[24].

SIRT2 regulates cell cycle[25], and among the non-histone target proteins, deacetylases tubulin in skeletal muscle[26], and FOXO1 in adipocytes[27], promoting cell differentiation. SIRT2 also plays a protective role in brain aging and neurodegenerative disorders[28].

SIRT3 regulates oxidative phosphorylation and fat oxidation[29,30], and activates the manganese-dependent superoxide dismutase (MnSOD or SOD2), providing mainly protection against oxidative stress[31]. SIRT4 promotes ADP-ribosylation activity which is responsible to regulate insulin secretion in pancreatic β-cells[32]. SIRT5 regulates urea cycle through its deacetylase activity on the carbamoyl phosphate synthetase 1[33].

SIRT6 contributes to telomere maintenance[34], and is also involved in protection against aging; it has been shown that lack of SIRT6 may induce an accelerated senescent phenotype in mice[35].

SIRT7 is still the less studied SIRT; some studies reported that lacking of this protein may be associated to acceleration of aging[36], and a strong link with this protein has been recently reported with protection against cardiorenal disease[37].

2.2. Uncoupling proteins

Uncoupling proteins are inner mitochondrial membrane proteins and belong to mitochondrial anion carrier family (MACP)[38,39], that is responsible for the uncoupling during the oxidative-phosphorylation in mitochondrial respiration, dissipating energy as heat (thermogenesis) [40]. The UCP family is composed by 5 members (UCP1-5). UCP1 was firstly described 30 years ago; it is expressed in BAT, murine and human WAT[41], and in human skeletal muscle[42]. UCP1 is up-regulated by cold exposure and high fat diet[43,44]. Ectopic skeletal muscle expression of UCP1 in mice, fed with high fat diet, improves glucose tolerance[45]. UCP1 reduces ROS production by reducing mitochondrial membrane potential[45].

UCP2 is widely distributed in a variety of tissues: BAT, WAT, brain, islet of Langerhans and does not play a role in thermogenesis[46–49]. UCP2 has a relevant role in regulating insulin secretion in pancreatic β-cells[50]; in fact, knockout mice for UCP2 have significantly increase in insulin secretion after glucose stimulation[50].

UCP3 is distributed in skeletal muscle of both rodents and human, and in BAT of rodents[38]. UCP3 plays a role in protecting against oxidative stress[51]: its overexpression reduces oxidative stress in murine myotubes subjected to hyperoxia, preventing oxidative stress-mediated muscle degradation[51].

UCP4 and UCP5 are mainly located in brain[52]. They exert a protective role against oxidative stress and neuronal death induced by hydrogen peroxide and dopaminergic toxins[52]. Genetic variants of *Ucp4* were described to be associated with schizophrenia and Amyotrophic lateral sclerosis (ALS)[52].

3. Vascular aging: Different phenotypes of atherosclerosis

VA is a gradual process involving biochemical, enzymatic, and cellular events in vascular area combined with epigenetic and molecular alterations. It is considered that atherosclerosis, a complex and multifactorial disorder playing a significant role in cardiovascular and stroke etiology[53], is a fundamental reflection of biological aging[54]. Now a day, CP, and STIFF are considered different phenotypes of atherosclerosis in the carotid artery, in association to the phenotype derived by arterial thickening, IMT [55]. Moreover, in the last decades an emergent role of vasa vasorum in the pathogenesis of atherosclerosis has been proposed [56,57].

IMT is defined as a double-line pattern between the intimal-luminal and the medial-adventitial interfaces of the carotid artery wall[58]. The intima and the media layer increase with aging as a result of biomechanical processes, such as blood flow and tension on the arterial wall [59], leading to the activation of molecular and cellular pathways involved in the formation of CP. IMT is considered a marker of arterial injury[60] and increased cardiovascular risk[61].

The Mannheim consensus defined CP as a focal structure encroaching into the arterial lumen of at least 0.5 mm or 50 % of the surrounding IMT value, or demonstrates a thickness >1.5 mm as measured from the media—adventitia interface to the intima—lumen interface[62]. CP presence, size[63], composition, plaque echodensity (echolucent vs. echogenic) and morphology are all important predictors of CVD[64]. Plaque echodensity is assessed using plaque image analyses techniques, such as gray scale median (GSM)[65]. Echolucent plaques are lipid-rich and present low GSM, whereas echogenic plaques are rich of fibrous tissue and calcification and present high GSM. Plaque echolucency is associated with a higher ipsilateral stroke risk due to plaque instability [66].

Although IMT and CP share the effect of some atherosclerotic risk factors, they have different natural history, associated risk factors, and prediction of vascular events. While IMT is mainly associated with hypertension, and media hypertrophy in the vessel wall[67], CP is more related to dyslipidemia and hypercholesterolemia. CP is strongly influenced by environmental factors, while IMT is strongly influenced by genetic factors[68]. Finally, in large population-based studies, CP is a stronger predictor of CVD compared to IMT[13].

Arterial stiffness is defined as the reduced ability of the vessel wall to adapt to the deformation induced by systolic blood pressure (BP) during the cardiac cycle[69]. Some authors report a linear relationship between arterial stiffness and age, while others reported accelerated stiffening between 50 and 60 years of age[70]. Hyperlipidemia, diabetes mellitus, elevated body mass index and smoking, are associated with accelerated STIFF[71]. However, when adjusted for age and blood pressure these findings are inconsistent in different studies[71].

IMT, CP and STIFF represent biologically and genetically distinct phenotypes correlated with atherosclerosis and may be the different

aspects of VA, even though they also can be dependent from mechanism not related with aging.

However, the diverse phenotypes of VA may develop differently across vascular anatomic location. For instance, presence of plaque and IMT are more often associated with large arteries in peripheral circulation, while stiffness and change in arterial compliance are more often associated to small vascular bed. These phenomena are manly linked to the structure of the vessels, since large arteries are rich in elastin and collagen, and small muscular arteries are rich in vascular smooth muscle. Obviously, based on the different genetic impact of phenotypes of VA, the same will results in a diverse predisposition of risk for atherosclerosis and vascular events [72].

4. Sirtuins and atherosclerosis

Oxidative stress and both systemic and endothelial inflammation, caused by turbulent blood flow, are associated with development of atherosclerosis[73]. SIRT1 protects against endothelial inflammation [74], and reduces endothelial wall damaged induced by oxidative stress [75]. An increase in SIRT1 expression was reported in mice's vessels with laminar vs. turbulent flow, suggesting a possible protective effect of SIRT1 against atherosclerosis[76]. SIRT1 transgenic mice (*sirt1*-Tg) hyper-expressing SIRT1, have less endothelial dysfunction compared to wild type animals[77]. After EX-527 administration, a specific SIRT1 inhibitor, *ApoE*$^{-/-}$ mice subjected to HFD, showed increase in plaque size and macrophages infiltration, suggesting as SIRT1 is strongly associated with protection against atherosclerotic plaque formation and growth[78].

The association between SIRT1 and atherosclerosis has been reported also in clinical studies. Coronary artery disease (CAD) patients show a downregulation in SIRT1 expression and activity in monocytes, linked with higher level of oxidative stress and pro-inflammatory state [79]. Nevertheless, SIRT1 overexpression, induced by SIRT1 activator (SRI1720), reduces both oxidative stress and inflammation[79], suggesting that SIRT1 could provide a direct anti-atherogenic effect offering novel therapeutic strategies for CAD care.

SIRT3 plays a relevant antioxidant effect by regulating mitochondrial function[80]. In a study conducted in mice knockout for LDL and SIRT3 (*Ldl*$^{-/-}$/*Sirt3*$^{-/-}$) the absence of SIRT3 significantly increased oxidative stress and risk for worse lipid associated outcomes[81]. The inactivation of Superoxide Dismutase 2 (SOD2) in mice model accelerates atherosclerosis progression[82]. Resveratrol, a natural polyphenol, enhances SOD2 activity and reduces oxidative stress, by activating SIRT3 in endothelial cell[83]. Reducing ROS generation in mitochondria is crucial to improve the efficiency of mitochondrial electron transport chain (ETC) and ATP production. Resveratrol increases SIRT3 expression in mitochondria, promoting transcription of several ETC-subunits [83], ultimately demonstrating that SIRT3 activation is responsible for maintaining redox homeostasis in endothelial cells, reducing the risk of CVD onset. Similarly to resveratrol, esculetin, another polyphenolic compound, increased SIRT3 mitochondrial levels[84], and induced mitochondrial biogenesis mediated by SIRT3 in human aortic endothelial cells (HAECs)[84]. These results confirm the anti-atherogenic role of SIRT3, by reducing the burden of mitochondrial metabolism and the subsequent oxidative state predisposing to CVD and atherosclerosis.

SIRT2 activation and overexpression stabilizes atherosclerotic plaques, by regulating lipid metabolism, and gluconeogenesis[85]. SIRT6 regulates level of LDL cholesterol in blood especially by inhibiting PCSK9 enzyme[86].

All these findings highlight the beneficial effects of sirtuins against cardiovascular diseases. Besides the evidences reported to modulate sirtuins, dietary restriction and in particular caloric restriction (CR), has been reported to exert cardioprotective effects via sirtuins activation [87]. In particular, in the recent interesting review by Wei and colleagues, it has been reported that CR not only may directly modulate the activity of sirtuins, but also indirectly; the metabolic adaptions related to CR, in fact, promote the release of small metabolites and non-coding RNA that can impact sirtuin activity and, in turn, cardiovascular function [87].

5. UCPs and atherosclerosis

UCPs, through Mitochondrial Oxidative Phosphorylation System (OXPHOS) dissipating energy as heat, reduce ROS production and increase mitochondrial respiration by reducing ROS-induced damage in endothelial cells[88]. Conversely, some studies report that OXPHOS uncoupling is indeed detrimental for endothelial cells and promotes atherosclerotic lesion progression[89,90].

Data generated on *ApoE*$^{-/-}$/*Ucp1*$^{-/-}$ mice, reported as absence of UCP1 is protective against plaque growth[91]. Moreover, exogenous adiponectin administration dramatically reduces UCP1 levels and, subsequently, plaque growth, suggesting that UCP1 strongly contributes to plaque formation and atherosclerosis progression[91].

UCP2 knockout (*Ucp2*$^{-/-}$) mice, similarly to *Ucp1*$^{-/-}$ mice, have a greater size of atherosclerotic lesions and macrophages infiltration compared to wild type animals[92]. Moreover, a significant increase in ROS production in absence of UCP2 is observed, suggesting that UCP2 exerts anti-atherogenic effect in vascular cells[92]. *Ucp2*$^{-/-}$ animals show a significant reduction in the activity of Glutathione peroxidase (Gpx), SOD, and catalase compared to wild type mice, both after chow and atherogenic diet, and develop more numerous and larger atherosclerotic plaque compared to control mice[93]. Taken together these data, suggest that UCP2 reduces susceptibility to atherosclerosis by lowering both oxidative stress and inflammation.

The presence of macrophages in the plaque is associated to plaque instability[94] and higher plaque temperature in patients with unstable plaque compared to stable plaque carriers, suggesting that reducing plaque temperature may reduce macrophages content and, subsequently, plaque instability[94]. UCP2 is the only member of the UCPs described in macrophages and that has a reported role in atherosclerotic plaque stability[95]. UCP2 is highly expressed in subendothelial macrophages of advanced plaques, and contributed to temperature heterogeneity[95].

The role of UCP3, 4 and 5 on atherosclerosis, at the best of our knowledge, is still under investigation.

6. Sirtuins, UCP, and vascular aging: Experience from NOMAS

6.1. Sirtuins, UCP and IMT

In order to investigate the association between SIRT and UCP proteins and IMT, we examined 1018 participants with IMT and genotype data from NOMAS. The mean age of the participants was 70 ± 9 years, 61 % were women, 67 % Hispanic, 17 % non-Hispanic Black, and 15 % non-Hispanic White. The mean IMT was 0.96 ± 0.10 mm for men and 0.93 ± 0.09 mm for women. This study manly reported as *rs12363280* in *Sirt3* was associated with decreased risk of elevated IMT, while rs1430583 and rs6818140 in *Ucp4* were associated with increased IMT [12]. Analyses for interaction between SNPs and sex showed a decreased IMT in women carrying the polymorphism rs3825075 in *Sirt3* and an increased risk of elevated IMT in men carrying the rs1430583 SNP in *Ucp1* [24]. Analyses that explored the interaction between SNPs and smoking status demonstrated an increased IMT in smokers that carry the polymorphism rs4802998 in *Sirt2* or the SNP rs7109266 in *Ucp2* [24]. At the best of our knowledge, no similar studies have been conducted so far, which investigated association between genetic variants of these proteins and this phenotype of VA.

6.2. Sirtuins, UCP and CP phenotypes

Large family studies have described as CP heritability is about 20–50 %, suggesting a significant contribution of genetics to this phenotype of

Table 1
Association between *Sirt/Ucp* SNPs polymorphisms and carotid plaque (CP) presence, number, total plaque area (TPA) and echodensity (GSM). 85 SNPs analyzed in the 11 *Sirt* and *Ucp* genes.

Gene	Chromosome	SNP	Sample size (stroke-free subjects)	Logistic (plaque presence) and Poisson (plaque number) regression models		Analyses for interactions of SNPs with sex, smoking status, diabetes and hypertension	
SIRTUIN							
Sirt3	11	***rs12363280***	1356	–	–	Increased **GSM** in men	G-carriers: **β = 12.53 (95 % CI = 3.88–21.17)**
		rs4980329		–	–		T-carriers: **β = 11.62 (95 % CI = 2.86–20.38)**
Sirt5	6	***rs4712032***	1018	Increased risk of **plaque number**	CP number: **RR = 1.14 95 % CI = 1.06–1.22)**	Increased risk of **plaque number** among non-smokers	CP number: **RR = 1.47 95 % CI = 1.20–1.82)**
		rs12216101			CP number: **RR = 1.16 95 % CI = 1.07–1.26)**	–	–
Sirt6	19	***rs107251***	1018	Increased risk of **plaque presence** and **number**	CP presence: **OR = 1.71 (95 % CI = 1.23–2.37)** CP number: **RR = 1.31 95 % CI = 1.18–1.45)**	–	–
		rs107251	1356	Increased risk of greater **TPA**	T-carriers: **β = 0.30 (95 % CI = 0.14–0.45)**	–	–
UCPs							
Ucp1	4	***rs1430579***	1018	–	–	Reduced risk of **plaque presence** in men	CP presence C-carriers: **OR = 0.80 (95 % CI = 0.72–0.90)**
		rs1430583	1356	–	–	Reduced **GSM** in women	T-carriers: **β = -8.87 (95 % CI = -14.35 - -3.39)**
Ucp3	11	***rs1685356***	1018	–	–	Increased risk of **plaque presence** in women	CP presence A-carriers: **OR = 1.42 (95 % CI = 1.08–1.87)**
		rs1685354	1356	–	–	Increased risk of greater **TPA** and **GSM** in participants with hypercholesteremia	TPA increase G-carriers: **β = 0.27 (95 % CI = 0.09–0.46)** GSM increase rs12363280 G-carriers: **β = 11.56 (95 % CI = 4.67–18.44)**
		rs2734827	1356	–	–	Increased risk of greater **GSM** in participants with hypercholesteremia	A-carriers: **β = 9.50 (95 % CI = 3.01–15.99)**
Ucp5	X	***rs5977238***	1018	Reduced risk of **plaque presence** and **number**	CP presence: **OR = 0.49 (95 % CI = 0.32–0.74)** CP number: **RR = 0.64 95 % CI = 0.52–0.78)**	–	–

atherosclerosis[96]. In 2 different studies conducted in 1356 stroke-free participants enrolled in NOMAS GWAS, we found that *Sirt3*, *5* and *6* and *Ucp1*, *3* and *5* were associated with different genetic contributions of CP; while *Sirt5* and *6* were associated with increased plaque presence and number, *Ucp5* seemed to have a protective role against CP presence and number [15]. Both SNPs rs4712032 and *rs12216101* in *Sirt5* were associated with increased number of CP after adjustments for several covariates such as age, sex, smoking and hypertension[15]. The SNP rs107251 in *Sirt6* was associated not only with increased risk of CP presence and number but also with greater TPA. Analyses for interactions of SNPs with sex showed that 2 SNPs (*rs12363280* and rs4980329) in *Sirt3* were associated with increased CP echodensity in men.

Ucp5 rs5977238 was associated with a reduced risk of plaque presence and number and two SNPs in *Sirt3* gene (*rs4980329* and *rs12363280*) in the association with mean GSM [14]. After analyses for interaction between SNPs and sex, 2 SNPs (*rs1430579* and rs1430583) in *Ucp1* were associated with reduced risk of plaque presence in men and lower GSM in women, respectively. Finally, rs1685356 polymorphism in *Ucp3* was related to increased risk of CP presence in women [15]. Analyses that looked at the interaction between SNPs and hypercholesterolemia showed that 2 SNPs (*rs1685354* and *rs2734827*) were related with greater TPA but also greater GSM in participants with hypercholesterolemia [14].

6.3. Sirtuins, UCP and STIFF

Similarly, to the other phenotypes of VA (CP and cIMT), STIFF is influenced by genetic factors[96]. We analyzed 1143 participants with STIFF and genotype data from NOMAS[13]. We reported the main role of SIRT1 and 5, and 3 UCP1, 3 and 5[13] in association with arterial elasticity. While *Sirt1* and *Ucp5* were associated with lower STIFF, *Sirt5*, *Ucp1* and *3* were associated with increased STIFF. SNP rs7895833 in *Sirt1* and *rs129308417* in *Ucp5* associated with decreased STIFF, while 4 different polymorphisms in *Ucp1* (*rs141698129*, *rs141704584*, *rs141706434*, and rs1707405), 1 polymorphism in *Ucp3* (*rs73397813*) and 1 SNP in *Sirt5* (*rs10498683*) associated with increased STIFF. Analyses that explored the interaction between SNPs and smoking status showed lower STIFF in smokers carrying the SNP *rs17712705* in *Sirt1*. Analyses for interactions of SNPs with diabetes demonstrated that 2 SNPs (*rs2253217* and rs9382227) in *Sirt5* were associated with increased STIFF among diabetic patients.

Table 2
Association between *Sirt/Ucp* SNPs polymorphisms and IMT. 85 SNPs analyzed in the 11 *Sirt* and *Ucp* genes.

Gene	Chromosome	SNP	Sample size (stroke-free subjects)	Logistic (plaque presence) and Poisson (plaque number) egression models		Analyses for interactions of SNPs with sex, smoking status, diabetes and hypertension	
SIRTUIN							
Sirt3	11	***rs12363280***	1018	Decreased risk of elevated **cIMT**	CC-carriers: **β = -0.041 (95 % CI = -0.082 - -0.001)**	–	–
		rs3825075		–	–	Decreased risk of elevated **IMT** in women	TT-carriers: **β = -0.026 (95 % CI = -0.049 - -0.002)**
Sirt2		***rs4802998***	1018	–	–	Increased risk of elevated **IMT** among smokers	GG-carriers: **β = 0.052 (95 % CI = 0.018–0.085)**
UCPs							
Ucp1	4	***rs1430583***	1018	Increased risk of elevated **IMT**	TT-carriers: **β = 0.036 (95 % CI = 0.010–0.062)**	Increased risk of elevated **IMT** in men	CC-carriers: **β = 0.062 (95 % CI = 0.024–0.100)**
		rs6818140			CC-carriers: **β = 0.035 (95 % CI = 0.010–0.060)**	Increased risk of elevated **IMT** among smokers	CC-carriers: **β = 0.035 (95 % CI = 0.010–0.060)**
Ucp2		***rs7109266***	1018	–	–	Increased risk of elevated **IMT** among smokers	AA-carriers: **β = 0.065 (95 % CI = 0.013–0.116)**

Table 3
Association between *Sirt/Ucp* SNPs polymorphisms and STIFF. 85 SNPs analyzed in the 11 *Sirt* and *Ucp* genes.

Gene	Chromosome	SNP	Sample size (stroke-free subjects)	Logistic (plaque presence) and Poisson (plaque number) egression models		Analyses for interactions of SNPs with sex, smoking status, diabetes and hypertension	
SIRTUIN							
Sirt1		***rs7895833***	1143	Decreased risk of greater **stiffness**	G/A-carriers: **β = -0.06 (95 % CI = -0.12 - -0.01)**		
		rs17712705		–	–	Decreased risk of greater **stiffness** among smokers	G-carriers DD: **β = 0.22 (95 % CI = 0.03–0.42)** SD: **β = 0.22 (95 % CI = 0.03–0.42)**
Sirt5		***rs10498683***	1143	Increased risk of greater **stiffness**	T/C-carriers: **β = 0.07 (95 % CI = 0.00–0.13)**	–	–
		rs2253217		–	–	Increased risk of greater **stiffness** among diabetic patients	C-carriers **β = 0.19 (95 % CI = 0.07–0.31)**
		rs9382227		–	–		T-carriers **β = 0.14 (95 % CI = 0.01–0.26)**
UCPs							
Ucp1	4	***rs141698129***	1143	Increased risk of greater **stiffness**	G/C-carriers DD: **β = 0.11 (95 % CI = 0.03–0.19)** SD: **β = 0.11 (95 % CI = 0.03–0.19)**	–	–
		rs141704584			A/G-carriers DD: **β = 0.10 (95 % CI = 0.02–0.18)** SD: **β = 0.10 (95 % CI = 0.03–0.18)**	–	–
		rs141706434			T/C-carriers DD: **β = 0.12 (95 % CI = 0.03–0.21)** SD: **β = 0.11 (95 % CI = 0.02–0.20)**	–	–
		rs1707405			G/A-carriers DD: **β = 0.12 (95 % CI = 0.03–0.20)** SD: **β = 0.11 (95 % CI = 0.02–0.20)**	–	–
Ucp3	*11*	***rs73397813***	1143	Increased risk of greater **stiffness**	A/G-carriers DD: **β = 0.11 (95 % CI = 0.00–0.22)**	–	–
Ucp5		***rs129308417***	1143	Decreased risk of greater **stiffness**	A/G-carriers DD: **β = -0.21 (95 % CI = -0.38 - -0.05)** SD: **β = -0.23 (95 % CI = -0.40 - -0.06)**	–	–

7. SIRT and UCP pathways linked to development of EVA and SUPERNOVA

As we demonstrated in NOMAS, variants of *Ucp* and *Sirt* genes were associated with variability in risk to develop VA.

As reported in Table 1, *Sirt3 rs12363280* increases GSM in men while, in the same population, decreases risk of elevated IMT in both sexes. In agreement, *Sirt3* rs3825075 protects more woman than men from risk of elevated IMT, suggesting different biological mechanisms beyond the development of GSM compared to IMT, especially among sex. *Sirt3* rs3825075 in people over and under 85 years of age from the "*Treviso Longeva (TRELONG)*" was associated with modulation of human longevity[97], suggesting that *Sirt3* genetic variability might be relevant for the modulation of age-related phenotypes of senescence. *Sirt3*-deficient mice model did not affect neither plaque burden nor features of plaque vulnerability (i.e., fibrous cap thickness and necrotic core diameter)[81]. However, loss of *Sirt3* was associated with accelerated weight gain and an impaired capacity to cope with rapid changes in nutrient supply, resulting in an increase of oxidative stress levels, expedited weight gain, and low metabolic adaptation from these mice [81]. The function of SIRT3 in EVA and SUPERNOVA can be more related to risk factors for vascular diseases than directly with biological mechanisms of atherosclerosis, as further demonstrated by its different role on IMT and GSM (see Table 2).

Sirt5 polymorphisms in NOMAS increased risk of plaque number, particularly among non-smokers. Similarly, *Sirt5* SNPs increased risk of greater STIFF especially among diabetic patients (See Table 3). *Sirt5* gene regulatory regions sequence variants, have been associated with increased risk for coronary atherosclerosis and then acute myocardial infarct in 381 Chinese patients[98]. The role of SIRT5 levels on EVA and SUPERNOVA, especially in vascular stiffness and risk for plaque phenotypes, was already suggested by its function on the association between mitochondrial oxidative stress production and apoptosis in vascular cells[99].

Sirt6 rs107251 increased both risk of plaque presence and number, and risk of greater TPA. In agreement with our study, significant and independent association of rs107251 C/T variant was found with presence of hypoechoic plaque, with an adjusted to sex, BMI, total cholesterol, hypertension, and smoking status[100]. *Sirt6* rs107251 is an intron variant, which might be in linkage disequilibrium with the neighboring functional variant[100]. A study conducted in 1749 Chinese participants recruited from various communities demonstrated as the presence of this SNP was associated with abnormal brachial ankle pulse wave velocity, an indirect measurement of atherosclerosis[101]. This result was obtained after adjustment for conventional environmental risk factors and after high level of soybean intake, suggesting a role of this genetic variant in the crosslink between gene-diet interaction and risk of atherosclerosis[101].

While *Sirt1* SNPs decreased risk of greater STIFF among smokers, in NOMAS the genetic variants of its homologous *Sirt2* increased risk of elevated IMT among smokers. *Sirt1* rs7895833 was associated with different risk for dyslipidemia in elderly Brazilian patients[102]. These findings associate levels of SIRT1 with vascular risk factors[73]. Most probably the impact of SIRT1on EVA and SUPERNOVA development is related to interaction with epigenetic mechanisms. Recently, using a system called "*ICE*" (inducible changes to the epigenome), it was innovatively demonstrated the pivotal role of SIRT1 and SIRT6 as chromatin-modifying factors[103].

Ucp1 rs1430583 increased risk of elevated IMT in men while reduced GSM in women; *Ucp1* rs6818140 increased risk of IMT among smokers, and several *Ucp1* SNPs increased risk of greater STIFF. It was already associated with vascular risk factors in European adolescents from the HELENA study[104]. Variants in *Ucp2* (*rs7109266*), similarly to *Ucp1*, increased risk of elevated IMT. Allelic variants in *Ucp2* rs7109266 (promoter −866G/A) was associated with different susceptibility to risk for diabetes in a Chinese Han population[105]. Subjects with AA genotype of rs7109266 in *Ucp2* had higher fasting insulin, HOMA-IR, and HOMA-β than subjects with different genotypes[105].

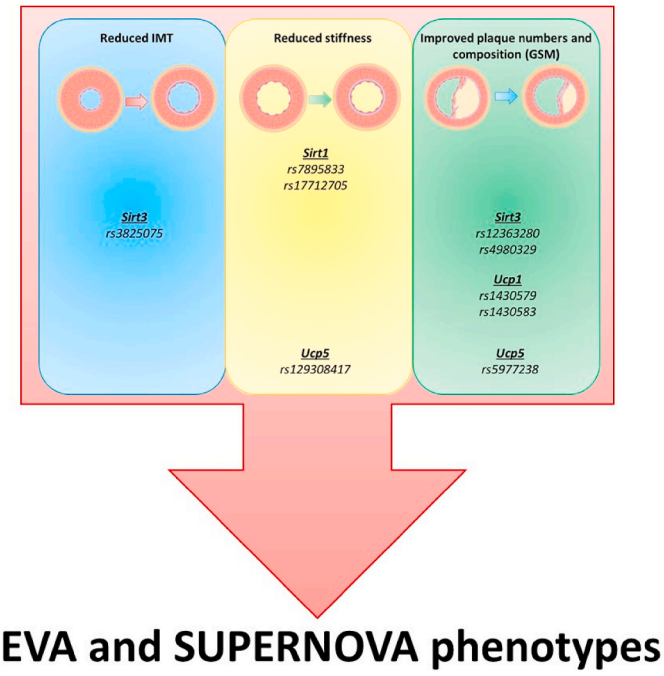

Fig. 1. Schematic representation for *Sirt* and *Ucp* related SNPs involved in EVA and SUPERNOVA. Created by BioRender.com.

As well as *Ucp1* and *2* variants, SNPs in *Ucp3* increased risk of plaque presence, greater TPA and GSM, particularly in women and in participants with hypercholesteremia. *Ucp3* rs1685356 and rs1685354 were associated with risk of obesity in 400 Dutch men between 40 and 80 years[106], and prediabetes in 2014 subjects from rural community in eastern China[107]. Moreover, *Ucp3* rs1685354 was linked with hand grip performances in elderly populations from Denmark, also influencing the survival patterns, with people carrying this allele showed higher mortality rates[108].

Instead, variants in *Ucp5* gene reduced risk of plaque presence and number and decreased risk of greater STIFF. We were able to find few information in literature regarding genetic polymorphisms in *Ucp5*. Interesting, variation in *Ucp5* was shown to interact with the APOE-ε4 carriers in the development of risk for neurodegenerative disease[109]. However, since predominantly in brain, the role of this gene/protein needs to be still fully clarified.

8. Conclusive remarks

SIRTs modulate UCPs expression directly[110], and also UCPs modulate SIRTs activities as positive/negative feedback, since UCPs in mitochondria control metabolic energetic state which depend the levels of SIRTs cofactors, like NAD[111]. Modulators of SIRTs activity were extensively proposed in science[112]. Recently, a new one referred as $A5^{+}$ (a mix of polyphenols and micronutrients), was reported to protect against metabolic and neurodegenerative diseases through activating SIRTs' pathways[112]. Physical activity, diet, polyphenols all are proven strategies to modulate SIRTs[113]. A recent study, by using "ICE" (inducible changes to the epigenome) system, demonstrated as the act of faithful DNA repair advances aging at physiological, cognitive, and molecular levels, including erosion of the epigenetic landscape, cellular ex-differentiation, senescence, and advancement of the DNA methylation clock, which can be reversed by epigenetic mechanism mediated by special factors including SIRT1, and SIRT6[103]. This innovation is epoch-making since, so far, we knew that we born with a certain risk to be an EVA or SUPERNOVA based on our genetic patterns,

however through lifestyle we can modify the probability to face vascular diseases in terms of modifiable risk factors. We know that our healthy lifestyle can also directly interact and change our DNA, and SIRTs and UCPs, and their genetic variants, are among the greatest candidates to be targeted to prevent and to protect against vascular aging (Fig. 1).

CRediT authorship contribution statement

David Della-Morte: Writing – review & editing, Writing – original draft, Validation, Supervision, Conceptualization. **Francesca Pacifici:** Writing – review & editing, Writing – original draft, Visualization. **Marialaura Simonetto:** Writing – original draft, Visualization. **Chuanhui Dong:** Writing – original draft, Formal analysis. **Nicole Dueker:** Writing – review & editing. **Susan H. Blanton:** Writing – review & editing, Visualization. **Liyong Wang:** Writing – review & editing, Visualization. **Tatjana Rundek:** Writing – review & editing, Validation, Supervision.

Declaration of competing interest

Declarations of interest: none.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.freeradbiomed.2024.05.010.

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