Chapter 2

Involvement of the Renin-Angiotensin System in Atherosclerosis

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Additional information is available at the end of the chapter

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Abstract

The renin-angiotensin system (RAS) is a well known for its role in the regulation of the blood pressure (BP). Angiotensin II (Ang II), the main mediator of the RAS, may act either, as a systemic molecule or a locally produced factor. Within the vessel wall it has significant proinflammatory role by inducing the oxidative stress, secretion of inflammatory cytokines and adhesion molecules. Ang II could trigger proliferation of vascular smooth muscle cells (VSMC) and its migration to the outer layer of the vessel wall. It could induce the release of matrix metalloproteinase (MMPs), from human VSMC and thus increase susceptibility to rupture of atherosclerotic lesions. Binding of Ang II to AT1R/AT2R could have opposing actions in vascular injury. The ACE2/Ang (1-7)/Mas axis of the RAS also opposes the unfavourable actions of ACE/Ang II/ATR1 axis. Inhibition of RAS could reduce inflammation-associated processes in vasculature, independently of lowering BP. RAS is significantly modulated by the genes coding for this system. Certain genetic variants (SNPs) in the RAS genes have been denoted as the functional ones and have been associated with hypertension, cardiovascular phenotypes and atherosclerosis. Also, the genetic components of the RAS interfere with the regulators of gene expression by microRNAs (miRs).

Keywords: renin-angiotensin system, atherosclerosis, genetic variant, micro RNA gene expression

1. Introduction

1.1. Short overview of the RAS

The renin-angiotensin system (RAS) is a cascade well known for its primary role in the regulation of blood pressure (BP) and sodium homeostasis. It has a significant role in regulating fluid and electrolyte balance by exerting its actions on the heart, blood vessels and kidneys.

The circulating RAS comprises liver-secreted angiotensinogen (AGT) that is enzymatically converted into angiotensin I (Ang I) in the bloodstream by kidney-derived renin. In the next step, Ang I is being converted by angiotensin-converting enzyme (ACE) to form Ang II. Ang II is the main effector in this system that acts either as a systemic molecule or as a locally produced factor.

The RAS is probably one of the most investigated biological systems over past 30 years. Given its pleiotropic biological effects, it is expected. Its complexity underlies the fact that research involving RAS molecules and actions in health and disease is still very active and intriguing. In the past decade, a substantial expansion of our knowledge of the RAS was emerged. It is verified by newly discovered components. One of them is a homologue of ACE, angiotensin-converting enzyme 2 (ACE2), which exerts a role as a negative regulator of the RAS [1] by cleaving Ang II to Ang-(1–7) [2, 3]. Namely, Santos et al. demonstrated that Ang-(1-7) is the ligand for the G-protein-coupled receptor Mas, and that the ACE2–Ang-(1–7)–Mas axis is the counter-regulating of the actions of classical RAS [4, 5]. Also, a variety of biologically active peptides, novel components of the RAS have been found recently: proangiotensin-12 (angiotensin-(1–12)) [6], angiotensin A (Ang A) [7, 8] and alamandine [9, 10].

1.2. Tissue and intracellular RAS

Our knowledge of the RAS has undergone substantial revision in the past few years. The existence of local (tissue) RAS systems that are independent of those stimulated by the classical RAS made it evident that the RAS is more complex than originally thought [11]. In that way, RAS is experienced substantial conceptual changes. Local (tissue) RAS represents tissue-based formation of angiotensin peptides that operate separately from the circulating RAS [12]. Tissue RAS systems are located in all major organs, including brain, heart, large blood vessels, adrenals and the kidneys [13]. Local RAS systems exert various actions depending on the type of cells involved and play crucial role in the maintenance of cellular homeostasis.

In order to identify a tissue-specific RAS at least one of the following criteria have to be fulfil [14]: (1) mRNAs for all components required for biosynthesis of a biologically active Ang II are present, (2) a biologically active angiotensin peptide is synthesized, (3) receptors for the biologically active angiotensin peptide are present, (4) the biologically active angiotensin peptide in the tissue is regulated, independently of the circulating RAS and (5) reduction or elimination of the action of the angiotensin peptide produces a physiological response.

There are other components of local RAS that are contributing to tissue-specific mechanisms of angiotensin peptide formation. They are participating in the progression of disease, or contrary, in mechanisms that protect from tissue injury [12]. These components include the (pro) renin receptor [15, 16], renin-independent mechanisms of Ang peptide generation from Ang-(1–12) [17, 18], intracellular RAS [19], previously mentioned ACE2/Ang-(1–7)/Mas receptor pathway [20] and they all may possess therapeutic potential.

Although different concepts of local RAS have been described, its key characteristic is a synthesis of AGT and enzymes, such as renin, that cleaves AGT to produce Ang I independently of the circulating RAS [12, 21, 22]. The presence of ACE, Ang II type 1 (AT1R) and type 2 (AT2R)

receptors and Ang II in different cells supports the concept of local RAS [23]. The local RAS seems to be regulated independently from the circulating system in a specific manner depending on the cell type and extracellular stimulus [24]. Despite that it can interact with the circulating system and complement it.

Some of the attempts to define local RAS that are independent of the circulating RAS were made in animal models [12]. One of the approaches to studying the functional importance of locally synthesized RAS components is to demonstrate their targeted overexpression or deletion in specific tissues. The evidence shows that in most tissues, local RAS enhances the actions of circulating Ang II, which has important implications for the pathophysiology of cardiovascular diseases.

In addition to classical and local tissue RAS, there is an intracellular RAS. This system is characterized by the presence of a functionally active RAS within the cells that can intracellularly synthesize Ang II [19, 25]. This means that Ang II is involved not only in an endocrine but also is a paracrine and an intracrine signaling system within tissues [26]. For example, intracellular delivery of Ang II leads to increase in intracellular calcium, growth of vascular smooth muscle cells (VSMCs) and regulation of muscle tone [27, 28]. This suggests that the intracellular Ang II has different functions compared to extracellular Ang II.

2. RAS and atherosclerosis

2.1. Molecular processes in atherosclerosis through the prism of RAS actions

Ang II, the main effector peptide of RAS, participates in all phases of the atherogenesis. It is proposed that the activation of RAS, and particularly Ang II, is involved in the initiation and progression of atherosclerosis in the absence of hemodynamic influences [29, 30]. Moreover, activation of RAS in the vascular wall has important modulatory activities in the development of atherosclerotic plaques, by stimulating a series of coordinated cellular and molecular events observed in the lesions.

2.1.1. Role of RAS in atherosclerosis development

The initial steps of atherosclerosis include endothelial dysfunction, which allows the migration of inflammatory cells and lipid droplets into the damaged part of the vessel wall, where they accumulate and form a "fatty streak". Oxidative stress is one of the main factors that promote vascular endothelial dysfunction. This is initial phase of vascular damage, when elevated levels of reactive oxygen species (ROS) that might be caused by Ang II induce impaired endothelial relaxation and vascular function [31]. ROS are free radicals involving oxygen, such as superoxide anions, hydroxyl radicals and hydrogen peroxide. These are mainly generated by mitochondria as by-products of cellular metabolism in the vessel wall by all vascular cells, including endothelial cells, VSMCs and adventitial fibroblasts. However, the imbalance between ROS generation and antioxidant protection leads to a state of oxidative stress, which can have deleterious effects as it modulates numerous cell signaling pathways. This is manifested as increased expression of pro-inflammatory genes, cell migration and proliferation, extracellular matrix production and apoptosis in the vessel wall, all of which play an

important role in vascular injury [32]. RAS activates NAD(P)H oxidase by enhancing Ang II/AT1R signaling which leads to increase in ROS production in both vascular endothelial cells and VSMCs [33, 34]. Ang II may traffic to mitochondria and AT1R could be expressed on outer mitochondrial membranes [35]. This way Ang II may stimulate an increase in mitochondrial oxidative stress, thus leads to VCMC senescence. Also, mitochondria may endogenously produce Ang II [36–38]. Several animal studies show that Ang II causes and contributes to aortic endothelial dysfunction [39–41]. It promotes abnormal vasomotion, a procoagulant state and transmigration of inflammatory cells into the vessel wall [42]. Within the vessel wall, Ang II increases vascular permeability via activation of vascular cell adhesion molecule-1 (VCAM-1) [43], intercellular adhesion molecule (ICAM)-1 [44], and endothelial growth factor (VEGF) [41, 42, 45, 46]. The key step in the formation of the initial lesion in atherosclerosis is the inflammation at the site of plaque formation caused by monocytes recruited from the blood stream by VCAM-1 [47]. Additionally, Ang II stimulates apoptosis of endothelial cell and VSMCs [48, 49].

The next stage in fatty streak formation is oxidation of low density lipoprotein (ox-LDL). Ox-LDL has important atherogenic properties as it penetrates the endothelial layer and gets taken up by macrophages and VSMCs, which results in the creation of lipid-containing foam cells. Ang II increases the interleukin-6 (IL-6)-mediated uptake of oxidized LDL by macrophages [50]. Moreover, Ang II upregulates lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and 12-lipoxygenase (12-LO) and 15-lipoxygenas (15-LO) expression in human VSMC. Thus, these two actions are accelerating LDL oxidation within the cell and enabling the internalization of exogenous ox-LDL, which could increase the susceptibility of human VSMC to transformation into foam cells [51].

The exposure of vascular cells to excess lipid (modified LDLs) with concomitant endothelial dysfunction/activation and the internalization and lipid deposits in the intima of vessel wall leads to further progression of atherosclerotic plaques [52]. Since advanced lesions predominantly consist of inflammatory cells, it is considered that at this stage progression of atherosclerosis is inflammation-driven. Modified LDLs enhance a broad range inflammatory responses, including activation, recruitment and infiltration of different immune cells (monocytes, neutrophils, natural killer cells, mast cells and dendritic cells) although the contribution of circulating monocytes is the most important [52]. When monocytes infiltrate and reach the sub-endothelium they differentiate into macrophages, under the stimulation of macrophage colony-stimulating factor (M-CSF). Macrophages are very adaptable cells that can undertake different phenotypes and functional characteristics, depending on the local microenvironment, which is a process known as ‘polarization’ [53, 54]. Distinct macrophage subtypes (M1 and M2) have been detected depending on the stage of atherosclerosis. Once differentiated, macrophages express high levels of pattern recognition receptors on their surface. These receptors have the ability to take up modified LDLs. Macrophages, then become lipid-laden and convert into foam cells. There is a potential role that Ang II provoking recruitment and activation of both macrophages and T cells into the vessel wall, by stimulating the expression of pro-inflammatory chemokines and cytokines, since both macrophages [55] and T cells express the AT1R on their surface [56, 57]. Ang II also increases monocyte chemoattractant protein-1 (MCP-1) expression in culture VSMCs as well as monocytes [58].

Importantly, Ang II induces the activation of several pro-inflammatory transcription factors. One of them is nuclear factor kappa B (NF-κB). Ang II activates NF-κB via AT1R in vascular cells and mononuclear cells, both *in vivo* and *in vitro* [59, 60]. The increase Ang II activates NF-κB by phosphorylating IκBα and p65 [61], which induces enhanced matrix metalloproteinase 9 (MMP-9) expression [62]. The AT1R mediates most of the actions of Ang II, but experimental data suggest that AT2R is also involved in Ang II-mediated NF-κB activation in inflammatory cell recruitment [63]. Recently, both an increase in AT1R and ACE levels and activation of NF-κB in heart have been reported in rat a model of a metabolic syndrome known as an inflammatory condition associated with accelerated atherogenesis [62, 64].

On the other hand, Ang II-induced activation of NF-kB could downregulate peroxisome proliferator-activated receptors (PPARs), PPAR-alpha and -gamma. This may diminish the anti-inflammatory effect of PPARs, thus contributing to enhanced vascular inflammation, leading to the acceleration of atherosclerosis in mice deficient for apolipoprotein E(ApoE -/-) mice [65]. Also, Ang II is inducing inflammation and remodelling of the vessel wall via activation of transcriptional mediator, Ets-1, member of ETS family of transcription factors [66]. Recently, inflammatory actions of Ang II were diminished by sirtuin-1 (SIRT-1) activator SRT1720. Treatment with SRT1720 decreased expression of TNF-a, IL-6, MCP-1, VCAM-1, ICAM-1, activation of NF-kB, STAT3 and infiltration of inflammatory cells in atherosclerotic plaques, induced by Ang II [67]. In order to inhibit Ang II signaling, SIRT-1 activation is a promising atheroprotective mechanism.

2.1.2. Role of RAS in atherosclerosis progression and acute complications

Over time continued plaque growth causes thickening and stiffening of the vessel wall and destabilizes it. This process results in a plaque rupture, which manifests as an occurrence of acute complications and development of ischaemic syndromes. Furthermore, the release of growth factors and cytokines by foam cells stimulates VSMC migration from the media into the intima. Upon arrival, these cells divide and produce extracellular matrix (ECM) components that contribute to the formation of the fibrous cap covering the plaque lipid core [68]. Also, Ang II triggers VSMCs to proliferate and migrate to the outer layer of the atherosclerotic plaques, where they produce growth factors and extracellular matrix proteins [69, 70]. The deposition of ECM components secreted by VSMCs in the plaques increases their size and eventually become occlusive. The interaction between exposed atherosclerotic plaque components, platelet receptors and coagulation factors from blood leads to platelet activation, aggregation and the subsequent formation of a thrombus, which may compromise the arterial lumen [52]. The thrombogenicity of the plaque is favored by a disturbance in the balance of coagulation and fibrinolysis. The role of Ang II, as a mediator of thrombogenesis has been also supported by animal studies [71, 72]. Namely, models of elevated Ang II levels, elicited both genetically and via chronic Ang II infusion, have demonstrated increased tissue factor (TF) expression and increased plasma plasminogen activator inhibitor-1 (PAI-1) level [73–75]. *In vitro* studies confirmed that Ang II induces the expression of TF in rat aortic endothelial cells [76] and human monocytes [77]. Chronic Ang II infusion induces platelet-endothelial cell adhesion [78] and accelerates thrombus formation in both large arteries [71, 79] and arterioles [74]. TF in atherosclerotic plaques initiates blood coagulation, directly stimulates SMC proliferation and activates

MMPs capable of degrading collagen. MMPs digest ECM scaffold, including the overlying fibrous cap, increasing plaque susceptibility to rupture. Moreover, Ang II induces release of MMP-2 in murine VSMCs via p47phox cytosolic subunit of the NAD(P)H-oxidase. Therefore, the activation of RAS contributes atherosclerotic plaque remodelling and potential destabilization via a NAD(P)H-oxidase-dependent activation of MMP-2 [80]. Laxton et al. demonstrated *in vitro* that MMP-8 cleaves Ang I to generate Ang II, and that MMP8-knockout mice have a substantial reduction in formation of atherosclerotic lesions [81]. Moreover, an association between MMP8 gene variation and extent of coronary and carotid atherosclerosis [81, 82] was observed. Significant upregulation of MMP-8 gene expression in carotid plaque tissue was observed in patients carrying haplotype G(-381)T(-799) of two MMP-8 promoter polymorphisms rs11225395 (-799 C/T) and rs1320632 (-381 A/G) [82]. Recently, it was shown that Ang II treatment *in vitro* causes increased collagen I synthesis and galectin-3 (Gal-3) expression in mouse HL-1 cardiomyocytes via protein kinase Calpha (PKC-α) pathway [83]. Gal-3 is involved in all processes active in atherosclerosis: cell adhesion, cell activation and chemoattraction, cell growth and differentiation [84]. An increase expression of Gal-3 mRNA in human carotid atherosclerotic plaque tissue may be affected by rare genetic variants of the haplotype block, previously associated with Gal-3 circulating levels [85, 86]. Diverse cellular processes in atherosclerosis are affected by microRNAs (miRs) and their expression are often tissue and disease-specific [87, 88]. Recently, the prediction algorithms and computational methods were applied to identify novel miRs important in pathogenesis of early and advanced atherosclerosis [89]. Amongst a number of miRs upregulated in atherosclerotic plaque, miR-155 shows dual properties in atherosclerosis and has particular interactions with RAS. Its activity could suppress Ang II-induced extracellular signal-regulated kinase (ERK1/2) phosphorylation and activation and regulate AT1R expression in different vascular cells [90, 91]. Moreover, it is shown that miR-155 downregulates AT1R expression, but not other RAS components [90].

2.2. Main RAS molecules in atherosclerosis through the magnifying glass

It is evident that Ang II, as a main mediator of RAS, promotes the formation of atherosclerotic lesions. In animal models of disease, AT1R deficiency in ApoE -/- and LDL receptor (LDLR-/-) atherosclerotic mice attenuates progression of atherosclerotic lesions, suggesting that AT1R mediates most of the Ang II functions [92, 93]. Hyperlipidaemia upregulates AT1R whose activation augments vascular oxidative stress and accelerates atherosclerosis [93], particularly as oxidized lipid becomes a neo-antigen that attracts components of the adaptive immune system to the vascular wall [94]. Consistent with this, AT1R deficiency causes a marked decrease in atherosclerotic lesion size in both the aortic root and arch of female and male mice, without a discernible effect on the composition. Also, aortic ATR2 mRNA expression is not altered in AT1R deficient mice, and AT2R deficiency is not affecting the lesion area or cellular composition [93].

Pharmacological inhibition of endothelial dysfunction and diet-induced atherosclerosis in ApoE AT1R-deficient mice dramatically attenuates the severity of atherosclerotic lesions [92, 95]. It is believed that the protective effects of the AT1R blockade with its antagonists (ARBs) include reduction of oxidative stress, reduction of inflammation and improvement in endothelial function [92]. Pharmacological blockade of AT1R reduces lipid accumulation and

increases the level of collagen within the atheroma and thereby stabilizes the formation of atherosclerotic plaques in ApoE-deficient mice [96] and in those with disrupted AT1R gene in bone marrow cells (BM) [97]. BM chimeric mice with disrupted BM AT1R show a reduced number of atherosclerotic lesions in the aorta and more stable plaques with reduced accumulation of BM-derived cells compared to AT1R-positive BM chimeric mice [97]. BM transplantation (BMT) from the ApoE-/-AT1R+/+ animals to the ApoE-/-AT1R-/- mice could restore Ang II-induced aggravation of atherosclerosis and plaque destabilization, even when the recipient's vascular cells do not express AT1R [98]. The contribution of AT1R in BM cells to the pathogenesis of atherosclerosis was demonstrated in LDL-receptor-deficient mice [99]. Hypertensive hypercholesterolemic ApoE-/- mice with either normal or endogenously increased Ang II production (renovascular hypertension models) were generated in order to study the contribution of Ang II to plaque vulnerability [100]. Staging and morphology of plaques significantly differed among these groups of mice and revealed an accelerated atherosclerosis in hypertensive animals. Plaques from mice with high Ang II appeared to be vulnerable, whereas plaques from mice with unchanged Ang II levels and similar blood pressure values were stable [100]. This mouse model of vulnerable plaque induced in a mouse is important and mimics a pathophysiological state commonly found in humans.

The expression of ERK1/2 and pro-inflammatory cytokines was reduced in supernatants of human carotid atheroma explant cultures treated with ARBs [101]. Also, in the same type of atheroma ATR1 blockade led to significantly reduced Ang II, MMP-1, MMP-8 expression and soluble elastin fragments [102]. This data recognized the ability of ATR1 blockade to modify plaque stability.

There are several beneficial effects assigned to the role of AT2R in atherosclerosis. AT2R overexpression in LDLR-knockout mice reduces atherogenesis in the aorta, as well as, expression and activity of MMP-2, MMP-9 and collagen accumulation in atherosclerotic regions [103]. In the same model, the presence of AT2R modulated oxidative stress, by decreasing expression of LOX-1, endothelial NO synthase (eNOS) and heme oxygenase-1 (HO-1) [104]. Also, in mice deficient for ApoE and AT2R on a diet rich in cholesterol, the atherosclerotic changes were exaggerated [105] which was shown as increased cellularity of atherosclerotic lesions [106]. After 16 weeks on a diet high in cholesterol, ApoE (-/-)/AT2R+ mice had significantly decreased a number of macrophages, VSMCs, lipids and collagen in the plaques due to apoptosis, compared to those deficient in AT2R gene [106]. Stimulation of AT2R by exogenous Ang II reduced atherogenesis in ApoE-/-/AT1R-/- double knockout mice [107]. It is evident that AT2R exerts atheroprotective effects when AT1R is inhibited. Vascular AT2R stimulation in transgenic ApoE-/- mice (AT2R-Tg/ApoE-/-) significantly reduces atherosclerotic lesion development in an endothelial kinin/nitric oxide(NO)-dependent manner and its anti-oxidative effect is likely to be mediated by inhibition of the superoxide-producing mononuclear leukocytes accumulation [108]. In ApoE-deficient mice, direct stimulation of AT2R by agonist CGP42112 improves endothelial function and stabilizes atherosclerotic plaques [109].

Evidence suggests that AT2R and ACE2, as a part of the ACE2–Ang-(1–7)–Mas axis, play a protective role in atherogenesis. Both factors have been detected within rabbit atherosclerotic plaques, AT2R and ACE2 immunoreactivity were observed in macrophages and alpha SMC

actin-positive cells [110]. ACE2 has been identified as a critical negative modulator of Ang II, counterbalancing the effects of ACE, by degrading Ang II and generating anti-atherosclerotic Ang-(1-7). Genetic ACE2 deficiency underlines vascular inflammation and atherosclerosis in the ApoE-/- mice [111]. Protective role of ACE2 and AT2R in cardiovascular pathology is supported by their decreased expression in male rat hearts on fructose-rich diet [112].

Also, ACE2 deficiency either in a whole body or in bone marrow-derived cells reduced atherosclerosis in LDLR-/- mice through regulation of Ang II/Ang-(1–7) peptides [113]. Overexpression of ACE2 in aortas of ApoE-/- mice transfected with AdACE2 (recombinant ACE2 adenovirus encoding full-length human ACE2 and co-expressing the GFP protein) led to less prominent macrophage infiltration than in aortas from control mice [114]. Also, overexpression of ACE2 enhanced plaque stability in a rabbit model of atherosclerosis [115]. Abdominal aorta segments transfected with AdACE2 showed a delayed onset of atherosclerotic lesions with fewer macrophages, less lipid deposition, more collagen contents, decreased expression of Ang II, MCP-1, LOX-1 and increased angiotensin (1–7) levels in plaque tissue [116]. In two different models of vascular disease, both hyperlipidaemia-induced atherosclerosis in ApoE-/- mice and mechanical injury-induced arterial neointimal hyperplasia in C57Bl6 mice, ACE2 deficiency resulted in significantly larger vascular lesions and neointimal hyperplasia compared with ACE2(+) controls [117]. ACE2 and exogenous Ang-(1-7) significantly inhibit early atherosclerotic lesion formation by preserving endothelial function and inhibiting of an inflammatory response in ApoE-/- mice [118, 119]. ACE2 activity and protein production were increased in atherosclerotic plaques treated with losartan *in vivo* in and *in vitro* in VSMCs [120]. Candesartan treatment restores vasoprotective and atheroprotective effects of the ACE2/Ang (1-7)/Mas receptor axis in high-cholesterol diet-fed ApoE-/- mice due to the inhibition of the pro-inflammatory-redox AT1R-mediated mechanism [121]. Increased ACE2 activation is considered to be a protective and compensatory mechanism that counterbalances ACE activity, and may play an important role in the treatment of atherosclerosis. Activation of ACE2/Ang (1-7)/Mas receptor axis by ACE2 activator (XNT) attenuates thrombus formation and reduces platelet attachment to vessels [122]. ACE2 overexpression in THP-1 (human acute monocytic leukemia cell line) *in vitro* decreases Ang II-induced MCP-1 production and this reduction is likely to be mediated by increased Ang (1-7) levels [123]. Blockage of endogenously activated Ang-(1-7) by chronic infusion of A779 attenuated late atherosclerotic plaque stability in high fat diet fed ApoE-/- mice [118]. All together ACE2 and Ang-(1–7) could be a therapeutic target for attenuation of atherosclerosis and the treatment of cardiovascular diseases.

3. Genetics of RAS in atherosclerosis

Over the past two decades, a large number of genetic investigations have been carried out to examine the association between genetic variants of RAS genes and vascular diseases, such as myocardial infarction, coronary artery disease and stroke. RAS genes were thoroughly associated with different risk factors for atherosclerosis, among which hypertension has a central role bearing in mind primary physiological role of RAS. Different cardiovascular phenotypes,

such as left ventricular hypertrophy, artery stenosis, artery stiffness and vascular remodelling were studied as well.

The story started with unforgettable discovery of ACE insertion/deletion (I/D) polymorphism (rs4340) associated with increased levels of ACE [124, 125]. This was the first discovery that implicated what is now fully accepted, that naturally occurring variations in DNA sequences, or polymorphisms (SNPs, insertion/deletions, copy number variations), mostly have the modifying effect in the development of atherosclerosis and together with gene-gene and gene-environment interactions are making an important contribution to the risk.

The most widely studied polymorphism in the RAS is I/D polymorphism, a287-bp Alu repeat element in intron 16 of ACE gene. It has been considered as a functional variant, since the ACE DD genotype was associated with higher circulating [124–126] and tissue mRNA levels of ACE [127, 128]. Among 78 variations that were found by ACE gene sequencing, 17 were in absolute linkage disequilibrium with the I/D polymorphism [129]. First genetic association studies were focused on ACE D allele effect on blood pressure [130, 131] and hypertension [132–134].

In atherosclerosis, most of the studies so far have been investigating ACE I/D polymorphism in association with subclinical and intermediate atherosclerotic phenotypes, such as intima-media thickness (IMT) with conflicting results. Meta-analysis of these studies uncovered moderate positive association of ACE D allele with common carotid IMT [135]. The association of ACE I/D polymorphism studies with advanced atherosclerosis has still been rare. As different mechanisms might be dominating the different stage of atherosclerosis development, as described previously in this chapter, it is always of importance to perform a genetic association study on early non-stenotic atherosclerosis and advanced stenotic atherosclerosis. A significant independent effect of DD genotype on plaque presence in patients with high-grade carotid stenosis (>70%) was noticed only in normotensive patients [136]. Another study failed to support the hypothesis that ACE genotype is a predictor of either the prevalence or the extent of atherosclerotic plaques but only in young adults [137].

Nevertheless, its role in atherosclerotic complications was noticed in a large-scale meta-analysis where the significant associations with ischemic stroke in approximately 18,000 cases and 58,000 controls were identified for four gene polymorphisms among which was ACE I/D [138]. An astonishing discovery was made recently, 23 years after Tiret et al. [124] found that ACE I/D influence on serum ACE levels. It was observed ACE expression appears to be regulated by mitochondrial uncoupling proteins (UCPs). Serum ACE activity was influenced by allele variants in UCP2 and UCP3 genes. This was the first evidence of association of serum ACE with a genetic variant outside the ACE gene [139]. This gave a new perspective on ACE investigation, suggesting that cellular feedback regulation might exist between ACE and UCPs. Even so, genetic variations in UCPs and SIRTs were recently associated with the atherosclerotic plaque existence [140] and morphology [141].

Also, both Ang II receptor genes, AT1R and AT2R, have many SNPs in the coding and its flanking regions, but the most studied are AT1R A1166C and AT2R -1332 A/G (+G1675A).

The A1166C polymorphism (rs5186) is located in the 3′ untranslated region (UTR) of AT1R gene. Primarily, it was investigated in association with hypertension but with inconsistent

findings. Association with hypertension was established in a certain subgroups of patients, e.g. only in subjects with severe, early onset, form of disease [142] and in long-term-treated subjects and/or with a family history of hypertension (HT) [143, 144] or in subjects with hypercholesterolaemia [145] or in males only [146]. A systematic review and a meta-analysis of the rs5186 variant failed to present sufficient evidence that polymorphisms in the AT1R gene are risk factors for hypertension [147].

Besides hypertension, rs5186 was associated with increased reactivity to Ang II in human arteries [148] and blood pressure response to exogenous Ang II [149]. In the context of atherosclerosis and different atherosclerotic phenotypes, previous studies addressed this polymorphism with inconsistent data. Some failed to show any significant effect for the A1166C polymorphism on mean IMT, carotid plaque formation [150] or internal carotid artery (ICA) stenosis [151]. The C-allele has been associated with a thicker carotid IMT in women [152] and increased IMT and IMT/D (common carotid artery diameter) ratio in hypertensive subjects [153]. A meta-analysis performed in 2011 suggests that the AT1R gene A1166C polymorphism is not associated with susceptibility to ischemic stroke [154]. However, the association between the AT1R 1166C allele and the presence of hypoechoic carotid plaques was recently found [155]. Confronting results could be attributed to differences in age, gender, belonging to different populations or ethnic groups, or different non-genomic and other external factors. The AT1R A1166C polymorphism is positioned in the target site for miR-155 [156, 157]. It was shown experimentally that human miR-155 downregulates expression of the 1166A allele alone [156], and that interaction between authentic miR-155 and the C allele is diminished, in a way that its ability to regulate AT1R gene expression is altered [157].

The AT2R, -1332 A/G polymorphism (rs1403543) located within the intron 1 of the gene was proposed to be functional, by affecting the mRNA alternative splicing and gene expression of AT2R. However, novel findings suggest that -1332 A/G might modulate protein expression, but not mRNA splicing [158, 159]. There are few studies that have been investigating this polymorphism in association with the presence of atherosclerotic plaques. Our study performed recently suggests that AT2R -1332 A/G polymorphism is a reliable gender-specific risk factor for carotid atherosclerotic plaque presence in females and could modify the inter-individual risk of cerebrovascular insult (CVI) among males with advanced carotid atherosclerosis [160]. It is still not clear which of the alleles, A or G, are more likely to carry a significant risk, even for hypertension and different cardiovascular phenotypes that were reproducibly investigated [161]. It was shown that a -1332 A/G polymorphism represents a risk factor for cardiovascular diseases and severe atherosclerosis by modifying systemic inflammation, especially in hypertensive males [162]. It is known that AT2R is expressed at low levels in the healthy adult vasculature. AT2R effects on cardiovascular structure and function may only become detectable under pathological conditions and/or after AT1R blockade. Expression of AT2R in human carotid atherosclerotic plaques was previously detected [163]. However, whether the stimulation of the AT2R is protective or deleterious in human atherosclerosis remains unresolved. The impact of AT2R during atherosclerosis or tissue injury should be studied by direct stimulation of AT2R to address potential therapeutic potential [164, 165].

4. Conclusion

Activation of RAS in the vascular wall has modulatory activities in the development of atherosclerosis by stimulating a series of cellular and molecular events. The balance between activation and repression of RAS could be decisive in the pathological remodelling, endothelial dysfunction and pathogenesis of atherosclerosis. Unfavorable and favorable effects of RAS molecules and their genetic variations, as well as consequently induced pathways, affect atherosclerosis development and following clinical events. This could have potential towards clinical application for risk stratification and therapeutics.

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