Chapter

The Renin-Angiotensin-Aldosterone System: Genomics, Proteomics and Therapeutic Implications

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Abstract

Since its discovery in 1898, the renin-angiotensin-aldosterone system (RAAS) has been intensely studied in the medical community, which led to important breakthroughs concerning the treatment of heart diseases. The main role of RAAS is to maintain the circulatory homeostasis, by maintaining the fluid volume. Angiotensin II (ANG II) can act on two receptors: angiotensin type 1 and angiotensin type 2 (AT1R and AT2R). The effect of AT1R consists in increased sodium retention, promotes vasoconstriction (mostly on the efferent arteriole), induces sympathetic nervous system activity, determines thirst and promotes the release of aldosterone. Abnormal activation of RAAS will determine hypertension and cardiac hypertrophy that may lead to heart failure. This is the reason why the pharmacological inhibition of this system has proven to induce such a beneficial effect in cardiovascular diseases such as hypertension and congestive heart failure. Later studies of patients with coronary artery disease revealed that angiotensin-converting enzyme (ACE) gene is also involved in the process of atherosclerosis and those mutations in its gene account for an increased susceptibility to severe acute coronary events. The most common ACE gene mutation is represented by deletions and insertions in the 16th intron (presence or absence of the 287-bp Alu repeat sequence), resulting in three possible genotypes, identified by the length of the fragments: II (490 bp), ID (490, 190 bp) and DD (190 bp). Scientific evidence suggests that the D allele plays a major role in the determination of coronary artery disease. The next step would be to develop new treatment strategies according to the genetic background of each patient.

Keywords: renin, angiotensin, aldosterone, sodium retention, hypertension, ACE gene mutations, D allele

1. Introduction

The renin-angiotensin-aldosterone system (RAAS) has long been discovered, since 1898 by Tigerstedt and Berman, but still possesses many mysteries to be solved. The RAAS has a major impact in controlling blood pressure and fluid balance.

The most important result of the activation of this system is the generation of angiotensin II (ANG II), which is a biologically active hormone that is produced through sequential cleavage of peptides derived from the initial substrate: the molecule of angiotensinogen. ANG II induces its actions after binding to different types of receptors and will induce a wide spectrum of biological reactions that can impact almost every system: the immune system, vessels, brain, heart and kidney.

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2. Renin-angiotensin-aldosterone system physiopathology

Renin is produced by the epithelioid cells of the juxtaglomerular cells under the form of a precursor named preprorenin. Afterwards it may be released as prorenin or may be processed to active renin, which will be stored in granules. The release of renin granules is the rate-limiting step of the renin-angiotensinogen-aldosterone cascade.

The next step consists in the release of angiotensinogen from the liver, which will be metabolized by renin, thus liberating angiotensin I (ANG I) [1, 2].

Angiotensinogen is included in the superfamily of Serpin A8 proteins. Serpin is a large and diverse superfamily of protease inhibitors and related proteins. It is released into the blood stream after removal of the 33-amino acid signal peptide and will remain in the circulation for approximately 5 h. However, it remains still unclear how much intact angiotensinogen versus catabolized angiotensinogen [the so-called des-(Ang I)-Agt] is present in the circulation. There is few data in the literature describing the proportions of intact Agt versus des-(Ang I)-Agt in the circulation. Other studies have suggested that des-(Ang I)-Agt may induce angiogenesis. Even though the liver is the main source of angiotensinogen synthesis, there are some other sources for this enzyme: the brain, heart, kidney, lung, adrenal gland, adipose tissue, blood vessels and digestive tube. An independent tissue regulation of angiotensinogen levels has also been proven [3–6].

ANG I will be transformed in ANG II due to angiotensin-converting enzyme (ACE), which is released from the endothelial cells. The angiotensinogen-converting enzyme is a dicarboxypeptidase that cleaves two amino acids from ANG I, thus generating ANG II (**Figure 1**).

There have been described two different types of ACE: somatic and testicular, which are both a result of the alternative splicing of a single gene. The role of ACE in forming ANG II from ANG I is primordial for its biological functions as ANG II is the major effector molecule of the RAAS. The ACE acts on other biologically active peptides, not only on ANG I, one of the most representative being bradykinin [7–9].

Bradykinin will be transformed into an inactive peptide through the action of ACE. This biological pathway for bradykinin metabolism is very significant in vivo. The ancient term for ACE is kininase II. As bradykinin promotes vasodilatation and induces a natriuretic effect, the pharmacological inhibition of ACE will diminish the kininase activity and will subsequently lower the blood pressure. The cleavage of ANG II by angiotensin-converting enzyme type 2 produces the heptapeptide angiotensin 1-7 (Ang 1-7). This peptide binds to the Mas receptor (MasR) and induces downstream vasodilatation, which has an opposing effect of the hypertensive action of AT1R signalling. The cardioprotective properties of Ang 1-7 can diminish or reverse heart failure and hypertensive cardiac remodelling [10] (**Figure 2**).
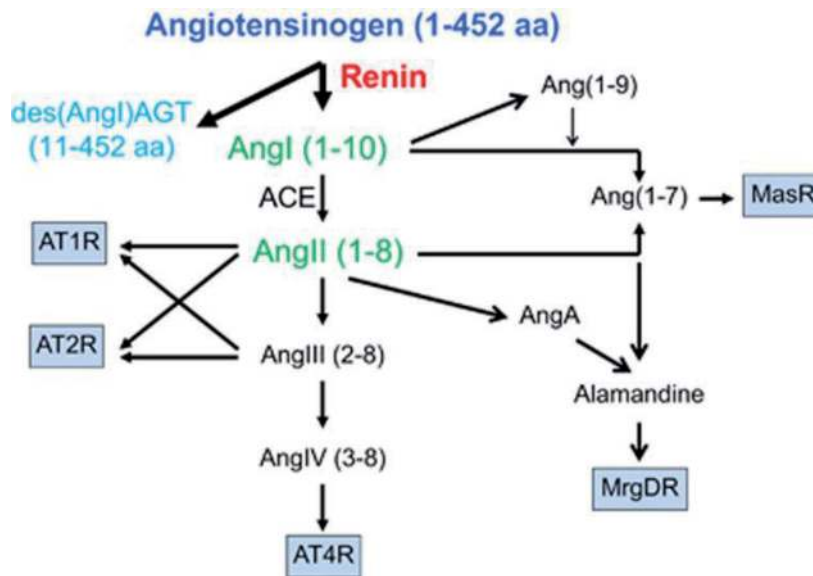
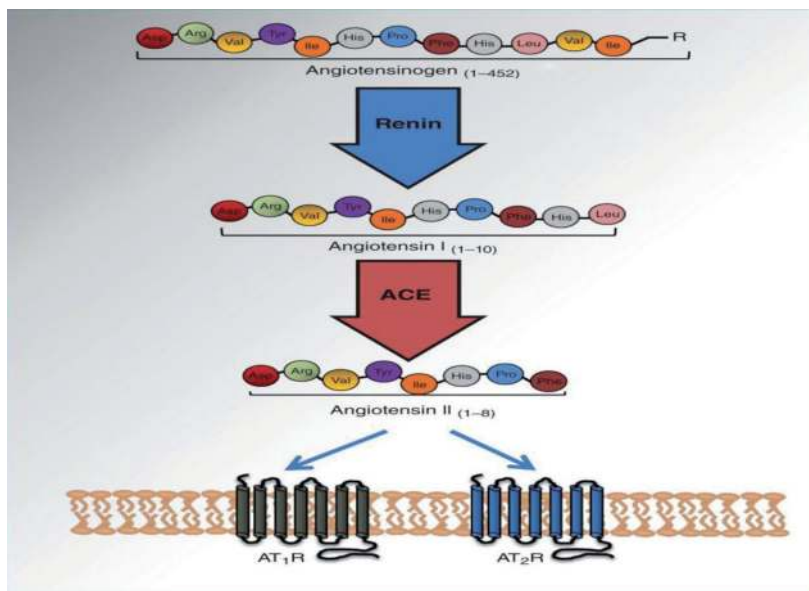**Figure 1.**
The renin-angiotensin-aldosterone cascade [3].

Figure 2.
ANG II formation [11].

ACE2 is a monocarboxypeptidase that transforms ANG I into Ang 1-9 which is a nonapeptide, and ANG II in Ang 1-7 (a heptapeptide). The discovery of these molecules unravels a distinct enzymatic pathway for ANG I and ANG II catabolism that will have an antagonist role to RAAS activation.

Ang 1-7 is a biologically active peptide that induces a wide range of effects, many of them being antagonist to those caused to Ang II. An endogenous orphan receptor, Mas (MasR), was identified in 2003; afterwards this receptor was proved to be Ang 1-7 receptor. It also protects cardiovascular function as it enhances vasodilatation via elevated release of NO and bradykinin, as well as diminishing the production of reactive oxygen species (ROS). The major effects of Ang 1-7 are those induced by MasR activation. These effects counterbalance the ones induced by ANG II and include the activation of the phosphatidylinositol 3-kinase

(PI3K)-Akt-endothelial nitric oxide synthase (eNOS) pathway, the inhibition of protein kinase C (PKC)-p38 MAPK pathways and the inhibition of collagen expression to limit cardiac fibrosis [12].

Also Ang 1-7 therapies have shown cardioprotective effects in preclinical models of non-ischemic and ischemic cardiomyopathy as it inhibited cardiomyocyte growth in vitro and diminished the ventricular hypertrophy induced by myocardial infarction in vivo. Ang 1-7 diminished the myocardial levels of pro-inflammatory cytokines (TNFα and IL-6), thus having a beneficial effect in cardiac inflammation.

Recent studies demonstrated that recombinant human ACE2 generated Ang 1-7 and Ang 1-9, while recombinant murine ACE2 generated predominantly Ang 1-7.

Ang 1-9 has also proved to be beneficial as it acts on AT_2R, thus leading to cardio protection. Therefore, the ACE2/Ang 1-7/MasR and ACE2/Ang 1-9/AT_2R axes are now considered to be physiological antagonists that inhibit the RAAS [10, 12, 13].

ANG II can act on two receptors: angiotensin type 1 and angiotensin type 2 (AT1R and AT2R). The effect of AT1R consists in increased sodium retention, promotes vasoconstriction (mostly on the efferent arteriole), induces sympathetic nervous system activity, determines thirst and promotes the release of aldosterone from the glomerular zone of the suprarenal glands (**Figure 3**).

The action on AT2R stimulation are antagonist to the one on AT1R as it induces vasodilatation and inhibits the inflammatory and fibrotic processes. This receptor is prevalent in the foetus; therefore, it has an important role in normal ontogenesis but has a minor role in adults [13–17] (**Figure 4**).

Aldosterone secretion results from the AT1R stimulation and is the final step of the RAAS. It is an important regulator of the hydric balance and of sodium and potassium exchange. The strongest inducers in aldosterone secretion are ANG II and the increased extracellular concentration of potassium, the synthesis of aldosterone being induced by CYP11B2 gene expression. Aldosterone will activate the mineralocorticoid receptor, leading to increased reabsorption of natrium and water in kidneys and promoting potassium excretion (**Figure 3**).

Besides the systemic RAAS, a local system found in many tissues, including the heart has been described. The local RAAS functions in a dual manner: independently and in correlation with systemic RAAS components. This second pathway of metabolisation present in the heart consists in ANG II synthesis by the endopeptidase chymase. Chymase is a serine protease found in a type of granulocytes called mast cells, which are located in the heart interstitium. Mast cells contain granules
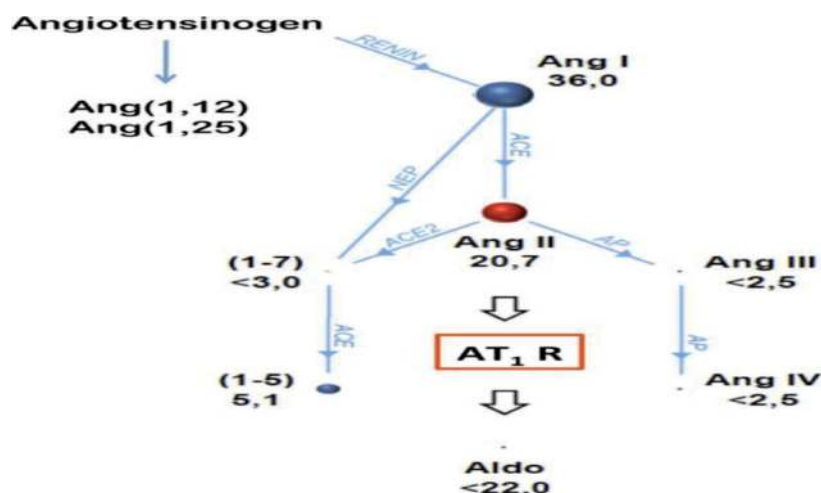

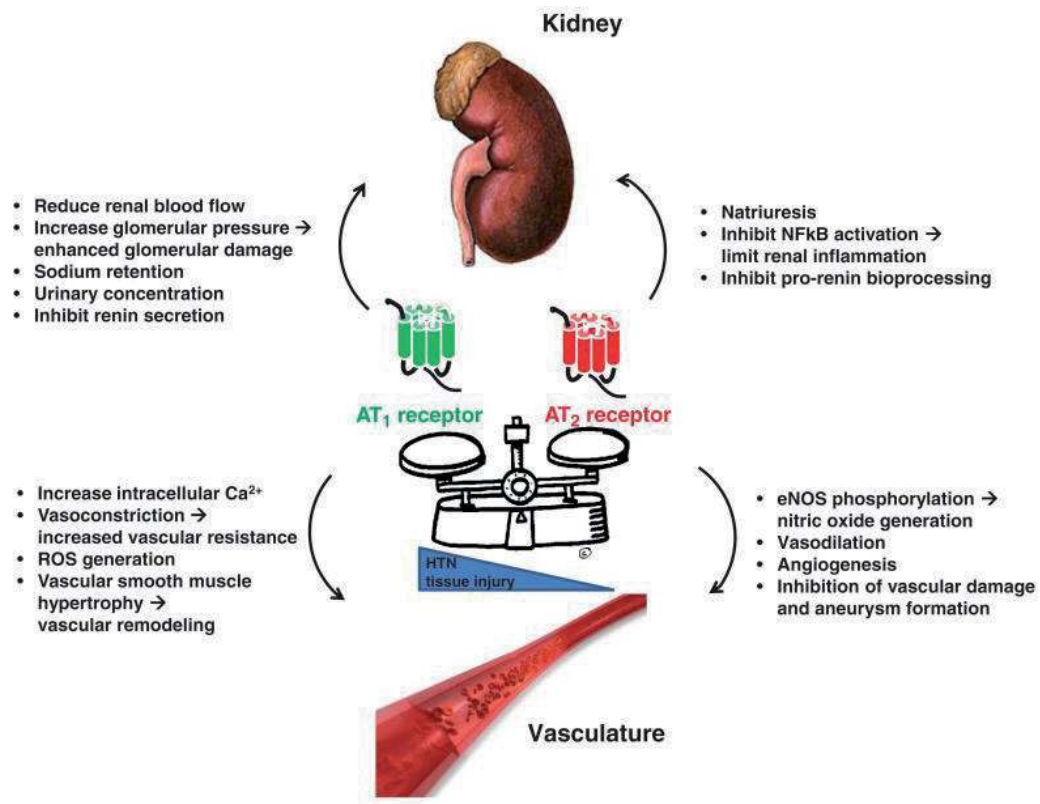**Figure 3.**
The RAAS activation [13].

Figure 4.
The actions of angiotensin receptors [11].

that are filled with cytokines and proteases (including chymase). The degranulation releases chymase during the inflammatory process via a classically mediated ligand-dependent pathway. Chymase actions are similar to ACE but with a much higher catalytic activity in transforming ANG I into ANG II. Therefore, chymase may be in fact a major ANG II-forming enzyme in the human heart. Its role may be linked to many pathological processes, such as hypertension, atherosclerosis, vascular proliferation, development of cardiomyopathies, myocardial infarction and heart failure, as well as cardiac fibrosis [18–21].

A third pathway consists in the action of ACE2 (monocarboxypeptidase angiotensin-converting enzyme 2), which is similar to ACE in <50% of amino acidic constitution and therefore is unresponsive to the treatment with ACE inhibitors. This enzyme transforms ANG I into Ang 1-9 and ANG II into Ang 1-7, thus promoting the synthesis of cardiorenal-protective peptides, through diminishment of ANG I and ANG II levels. This pathway may lead to a key role in modulating ANG II actions in cardiovascular disease [22].

3. Gene involvement

3.1 ACE gene polymorphisms and heart disease

The implication of gene alterations in the ethiopathogeny of different diseases is an important study direction in the later years. The RAAS is not only involved in the cardiovascular homeostasy but also in the development of the coronary artery disease. It seems that angiotensin-converting enzyme gene mutations have the key role in this process.

ACE gene is found on the long arm of chromosome 17, position 23 (17q23), and include 26 exons and 25 introns (**Figure 5**) [23].

There are several possible mutations of this gene which initiate cardiovascular disease. The most common one is represented by deletions and insertions in the 16th intron (presence or absence of the 287-bp Alu repeat sequence), resulting in three possible genotypes, identified by the length of the fragments: II (490 bp), ID (490, 190 bp) and DD (190 bp) (**Figure 6**).

Mutations are determined by collecting 2 ml of blood from the patients, extracting the DNA from the leukocytes, followed by amplification of the genetic material, agarose gel electrophoresis in order to separate the fragments and UV light identification of coloured fragments according to their length.

Those genotypes interact with the conventional environmental factors and influence the severity of the coronary artery disease. Most of the studies have demonstrated that the D allele is a risk factor, while the I allele is a protective factor. This hypothesis was first formulated by Cambien et al., in the study published in Circulation in 1994 [24]. They postulated that D allele is correlated with high plasma levels of ACE and that genotypes ID and DD increase the predisposition for myocardial infarction. Since then, this theory was largely debated. Guney et al. performed a study on 203 patients with acute coronary syndrome or positive functional tests, selected based on the severity of the stenosis (>70%), as revealed by the coronary angiography. The control group was formed of 140 patients with nonsignificant coronary stenosis. Results showed a higher prevalence of the D allele in the patient group than the control group ($p = 0.002$), in patients with hyperlipidaemia ($p = 0.009$) and smoking habit ($p = 0.004$), and a higher number of diseased vessel (two/three vessel diseases) ($p = 0.002$). The conclusion drawn was that D allele interacts with conventional risk factor and determines the degree of severity, since patients with D allele have higher plasma levels of ACE and are therefore more exposed to angiotensin II.

Dhar et al., in a study on 217 patients with coronary disease compared to 255 control patients (with negative Treadmill test), also found the D allele as an independent risk factor in coronary vessel disease ($p < 0.05$). Most of the ID and DD patients were heavy smokers, diabetic and dyslipidemic, suggesting the interaction between D allele and risk factors. Another association pointed out by other studies is that D allele is more frequent in obese patients. The high levels of angiotensin II might lead to disturbances in macronutrient oxidation, causing fat deposits and weight gain [25, 26]. Despite the fact that most of the studies focusing on this mutation were positive, results are still controversial, since there are a few studies which showed negative results. Poorgholi et al. concluded from their 1050 CAD patients' study that I/D polymorphism is not a predisposant factor [27]. Fujimura et al. also found no association between ACE gene polymorphism and CAD in his study on 1840 patients (947 ischemic patients and 893 healthy controls) [28]. Although in some populations, there were no positive correlations between D allele and coronary disease; this may be due to geographical differences and ethnical differences. In general, D allele seems to be a predisposing factor, and probably the severity of the ischemic heart disease (infarction or unstable angina, one/two/three
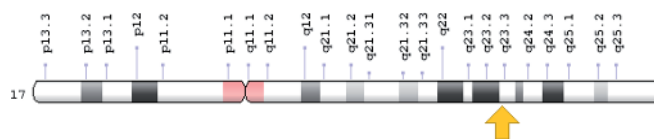

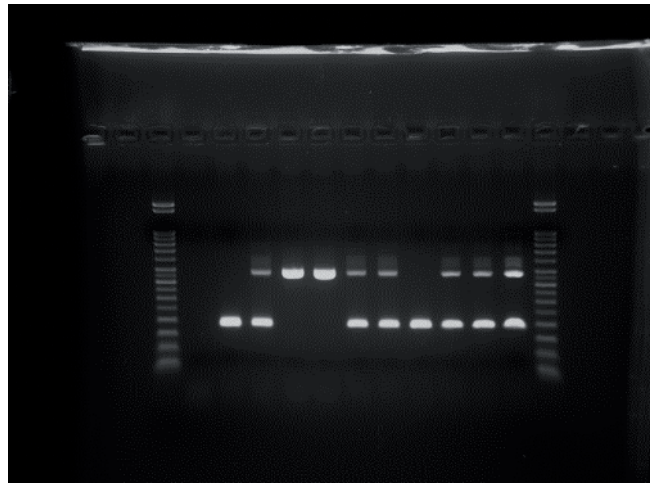**Figure 5.**
The ACE gene location [29].

Figure 6.
ACE genotypes: II (490 bp), ID (490, 190 bp) and DD (190 bp) [30].

vessel diseases) depends on the interactions between this mutation and the other risk factors: hypertension, diabetes mellitus, obesity, smoking habit and dyslipidae-mia. In most studies, patients with DD genotype were hospitalized for myocardial infarction and had multiple vessel disease and a high exposure to the conventional cardiovascular risk factors [31, 32]. The role of D allele in atherosclerosis was generally studied by measuring and analysing the carotid artery intima-media thickness (IMT). Results were statistically significant for high-risk patients, rather than low-risk patients. Therefore, D variant carriers have an increased risk of atherosclerosis, but only if they are also exposed to other genetic or environmental risk factors [33].

The association between ACE gene mutations and hypertension is explained by high plasmatic levels of ACE and a consequently increased quantity of ANG II in D-allele carriers. However, the predisposition for preeclampsia in D-allele women is less understood, and it is just a hypothesis still under debate. Preeclampsia implies high blood pressure and proteinuria after 20 weeks of pregnancy. It is the consequence of many risk factors, both genetic and environmental, and the RAAS may play a key role, since inactivating this system leads to a failure in achieving the hypervolemic status needed during pregnancy [34]. The studies on pregnant women are rare and often very small, making the available data on this issue very inconsistent. Some evidence suggests that it may come as a result of an abnormal regulation of the RAAS system and high intrauterine artery resistance [35]. Abedin et al., in a study on 296 patients, found no association between I/D polymorphisms but demonstrated a positive correlation with another ACE gene mutation, ACE rs4343, which was significantly present in the case group versus control group ($p = 0.0001$) [36]. On the other hand, a large meta-analysis including 45 studies found that DD and ID pregnant women have a higher risk of pregnancy-induced hypertension than II patients (74% D allele in the case group versus 56% D allele in the control group) [37]. Still, the large heterogeneity of the included patients lowers the force of the obtained results.

Another line of study in this field is represented by the involvement of RAAS system in the development of hypertrophic cardiomyopathy. This condition requires an anterior interventricular septum larger than 13 mm or a posterior interventricular septum larger than 15 mm, in the absence of hypertension and valvular disease. In most cases, hypertrophy is asymmetric. The implication of sarcomere protein gene mutations in the physiopathology of this disease is widely acknowledged. Later studies bring into attention a new puzzle piece in this genetic disorder, suggesting

that ACE gene may also be involved, since the inhibition of ACE reduces cardiac hypertrophy and remodellation post-myocardial infarction [38, 39]. Some studies suggest that ACE gene mutations explain the intrafamilial phenotype differences in hypertrophic cardiomyopathy, which is considered a monogenic disease [40].

3.2 ACE polymorphism and treatment response

Genetic background often explains the different response to medication of patients with the same medical conditions.

"Sodium sensitive" hypertensive patients have certain physiopathological particularities: enhanced activity of the sympathetic nervous system, reduced renal excretion of sodium, hypersensitivity to vasoconstrictive hormones (adrenaline, ANG II) and alterations of the counteracting atrial natriuretic peptide (ANP) [41]. The connection between salt and hypertension is still under investigation, and it has not been fully explained [42], although it is clear that for some hypertensive patients, the mean blood pressure is higher when salt ingestion is increased. Studies focusing on determining the frequency of sodium sensitivity in hypertensive patients revealed the following distribution: 51% high sodium sensitivity, 33% intermediate sensitivity and 16% sodium resistance [43]. Patients with D allele display a higher sensitivity to dietary sodium intake, and therefore the risk of developing hypertension in those patients is increased, when high-sodium/low-potassium diet is associated. However, when the diet is low in sodium, the risk between presence and absence of D allele seems to be the same [44]. ACE inhibitors represent a gold standard therapy for cardiovascular and renal disease. But almost half of the hypertensive patients respond to ACE inhibitors alone. Most patients require multiple drug associations. Previous studies analysed the role of ACE gene polymorphisms in modulating treatment response in different populations around the world, and the results are still inconsistent. Heidari et al. in their study on 72 newly diagnosed hypertensive patients revealed a stronger response to enalapril and lisinopril in the DD genotype than ID (p = 0.03) and II (p = 0.001) [45]. Arnett et al. demonstrated a greater response to hydrochlorothiazide in hypertensive women during a 4-week study [46]. Results are still controversial. Other studies found no relationship between RAAS inhibitory treatment and genetic polymorphisms. For example, the study performed by Millions et al. did not link the D allele with a supplementary reduction of blood pressure during the treatment with ACE inhibitors [47]. Despite the results of some important trials stating that DD patients have a better response to ACE inhibitors, those results turn out non-reproducible [48]. Moreover, the pharmacogenomic analysis from PROGRESS study (5685 patients) based on the neuroprotective effect of perindopril after stroke revealed no implication of the ACE polymorphism on the end points [49]. There is also some evidence pointing out a different response to hydrochlorothiazide according to this polymorphism and sex: diuretic treatment was more efficient in II women and DD men [50]. Others suggested the synergism between II, ID and a-adducin Gly/Trp as an even better predictor of response to diuretic treatment [50]. The importance of the polymorphism in salt-sensitive population, the size of the study population and the ethnic variability may partially account for those differences. The differences in the response to ACE inhibitors between Caucasians and Africans are overwhelming [51], and this definitely represents a solid motivation for further investigations in this field. As for the response to beta blockers, a very important piece in the hypertension puzzle, it seems that ACE gene mutations might also be involved. A randomized controlled trial in hypertensive patients treated with atenolol demonstrated a more important blood pressure decrease in patients with an AGT M 235 T or G-6A genotype. But those results were not reproducible [48].

Pharmacogenomics is a new medical field trying to achieve personalized treatments according to our genetic package, in an attempt to obtain better results in the treatment-resistant patients. Given that RAAS is a key system in cardiovascular haemodynamic, discovering mutations that explain individual differences in efficacy and toxicity of various drug categories, as well as designing treatment strategies taking genetic data in consideration, will certainly be a revolutionary breakthrough in the future, both for hypertension and coronary artery disease.

4. Renin-angiotensin-aldosterone therapeutical blockade

The blockade of RAAS has become a central therapeutic strategy for patients with cardiac pathology. The inhibition of RAAS system is realized with modulating drugs such as ACEi, angiotensin receptor blockers (ARBs) and mineralocorticoid receptor antagonists (MRA) [52, 53].

The direct renin inhibition is obtained upstream of the action of the converting enzyme and has been well-known for decades. This type of blockade prevents the generation of ANG I and subsequently ANG II. The most used drug is named aliskiren; it has been evaluated in two clinical heart failure trials: ASTRONAUT and ATMOSPHERE. These trials proved that aliskiren failed to reduce cardiovascular death or heart failure hospitalization, but it induced a significantly more important decrease from baseline in NT-proBNP levels [54].

One of the latest advances in RAAS is represented by the development of innovative AT1 receptor blockers which have a dual action, going beyond simple antagonism of the binding of ANG II. Similar to the classic ARBs, these molecules will act on the superfamily of G-protein-coupled receptors (GPCRs). When GPCRs are activated by an agonist, they will determine intracellular dissociation of a heterotrimeric G protein into G and G subunits. The result consists in inducing second messenger-mediated cellular responses. Other groups of proteins which induce specific signalling pathways in a manner that is independent of the protein G are represented by the 13-arrestins [52, 54].

Even though efficient blockade of RAAS system can be obtained at different levels, the cornerstone therapy remains the use of angiotensin-converting enzyme inhibitors (ACEi), ARBs and MRA. RAAS blockade combined with natriuretic peptides augmentation is considered to be a revolutionary treatment in heart failure patients.

The renin-angiotensin-aldosterone system and the natriuretic peptides have a yin/yang relationship that has a great potential in inducing beneficial effects, by influencing both systems. The desired effects of the RAAS blockade can be enhanced by augmenting the natriuretic peptides activity. Even though the monotherapy with neprilysin inhibitors has failed to prove efficiency in heart failure patients, the association with a RAAS blocker has overcome the disappointing results, and even more, it has proven to be one of the most promising therapies in patients with different types of heart failure [55–57].

5. Conclusions

The renin-angiotensin-aldosterone system is a key piece in the puzzle of cardiovascular homeostasis and disease. Therapeutical targeting of this system at different levels represents an important medical breakthrough. The observation that patients with the same condition and exposure to risk factors may have different disease evolutions and responses to treatment led to genetic studies and to the discovery

of the importance of ACE D allele in the determinism of coronary artery disease, arterial hypertension and hypertrophic cardiomyopathy. Therefore, developing treatment strategies according to the specific genetic pattern of the patient is the next step in medical evolution.

Conflict of interest

The authors declare no conflict of interest.

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