



# Genetics association of renin-angiotensin system with atherosclerosis

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## Abstract

Angiotensin-converting enzyme (ACE) gene encodes ACE, an enzyme of the renin-angiotensin system (RAS) that plays a significant role in homeostasis and control of blood pressure. It seems that genetic variations can affect ACE function and cause atherosclerosis, one of the most common diseases worldwide, especially in developed countries and one of the leading causes of death in these countries. The presence of ACE insertion/deletion (I/D) affect the function of ACE. This polymorphism can lead to progression of atherosclerosis in some population. Directory of Open Access Journals (DOAJ), Google Scholar and PubMed of the most common diseases worldwide. Additionally, EBSCO and Web of Science have been searched. In this review, we studied the effects of ACE I/D polymorphism in various populations and its influence on the risk of onset and progression of atherosclerosis. Depending on the population and the area under study, polymorphisms can contribute to the risk of disease.

**Keywords:** Genetic, Renin-angiotensin-system, Atherosclerosis

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## Introduction

The angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism can affect the plasma level and activity of ACE. Increased level of the ACE and ACE activity lead to atherosclerosis by raising the production of angiotensin II. In this review, the focus has been conducted on the study of the mechanism of atherosclerosis progression and the effect of different polymorphism in disease.

## Materials and Methods

Directory of Open Access Journals (DOAJ), Google Scholar, PubMed, EBSCO and Web of Science were searched with keywords relevant to atherosclerosis, coronary artery disease, RAS, ACE I/D polymorphism, ACE activity.

## Results

### Cardiovascular disease risk factors

The main risk factors are age, gender (male), high blood pressure, diabetes mellitus, hyperlipidemia, smoking, family history of coronary artery disease (CAD) and obesity. These risk factors can cause disease by increasing oxidative stress. In addition to these standard risk factors, it seems that genetic factors also play a role in CAD (1).

### Atherosclerosis

Coronary artery disease is one of the main causes of mortality and disability, in developing countries (1). The most important cause of CAD is atherosclerotic coronary artery obstruction (1). Atherosclerosis is known as an inflammatory disease that can affect the vessels of any part of the body (2). The occurrence of atherosclerosis in the coronary arteries is known as CAD. Accumulation of low-density lipoprotein (LDL) in the intima is an important step in atherosclerosis initiation (3).

### Mechanism of atherosclerosis

In the intima, the LDL particles convert to oxidized low-density lipoprotein (oxLDL) because of reactive oxygen species (ROS) (4). OxLDL binding to its specific receptor leads to an increase in chemokines, adhesion molecules and vascular cell adhesion molecule 1 (VCAM-1) level. The chemokines attract leukocytes to the injury. Activated endothelial cells express E and P selectins which bind to leukocytes and make them roll (5). After that, a firm adhesion happens via VCAM1, and finally, leukocytes enter the vessel wall. Endothelial and smooth muscle cells secrete the macrophage colony stimulatory factor that differentiate monocytes into macrophages (6).

Macrophages attract the oxLDL particles with their

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### ■ Implication for health policy/practice/research/medical education

Angiotensin II is an atherogenic factor that increases the production of adhesion molecules and foam cell formation in macrophages that lead to atherosclerosis.

receptors such as scavenger receptor A (SR-A) and finally turn into foam cells that eventually develop apoptosis (7). In this area, a set of cholesterol and apoptosis-residual cells form a necrotic nucleus. Due to the secretion of the matrix metalloproteinases (MMPs) in the area, this fibrous cap decomposes gradually and the necrotic nucleus enters the bloodstream. Then the plaques activate in that site and the end, these processes lead to vascular occlusion (8).

### *Molecular mechanisms of atherosclerosis*

Atherosclerosis is considered an inflammatory disease (1). Recent evidence suggests that several molecular pathways are involved in inducing and developing inflammatory responses to atherosclerosis. It seems that the RAS system plays a key role in the pathogenesis of atherosclerosis through inducing a set of molecular events observed at the site of injury (2).

Nowadays it is well-known that angiotensin II has an important pro-inflammatory effect in the vascular wall, which is the cause of atherosclerosis progression. There are two types of angiotensin II receptor in mammals, angiotensin receptor type1 (AT1R) and, angiotensin receptor type2 (AT2R) (9). Although both AT1R and AT2R receptors are found in the vessels wall, it seems that AT1R is responsible for angiotensin II atherogenic functions (5).

In the vascular wall, ACE is easily detectable in endothelial cells and smooth muscle cells. Therefore, RAS compounds are detectable in the vessels. RAS is locally activated in atherosclerotic plaques and damaged vessel (9). These results indicate that, in addition to activating the AT1R, the local activation of this pathway can also contribute to the onset of atherosclerosis (5).

Angiotensin II can increase the expression of adhesion molecules, chemokines and cytokines. These molecules interfere with endothelial cells function, oxidizing and absorbing LDL (10) and smooth muscle cells proliferation (2). In advanced atherosclerotic injuries, Angiotensin II induces MMPs and plasminogen activating inhibitor which is involved in the instability of the atherosclerotic plaque. Angiotensin II also increases the expression of vascular endothelial growth factor, which is involved in angiogenesis (2).

Informing and developing atherosclerotic plaques, RAS is activated locally and increases the expression of adhesion molecules and MCP-1. These molecules accelerate the use of inflammatory cells in the vascular wall (10).

Although vascular endothelium acts as a barrier and creates a non-adherent surface against leukocytes, in

conditions of disease, due to the induction effects of angiotensin II, the expression of adhesion molecules and inflammatory cells, inflammatory cells pass through the barrier. Monocytes are converted into macrophages after migration to the vascular wall, causing lipid deposition in the plaque (10). Monocytes/macrophages secrete chemokines and MMPs that accelerates plaques formation.

Leukocytes have NADPH oxidase that can act as a source of ROS. Therefore, activated RAS causes a link between circulatory leukocytes and vascular cells, which is an important step in atherosclerosis pathogenesis (11). Increased levels of ACE and angiotensin II have been observed in atherosclerotic plaques. In human atherosclerotic plaques, ACE, angiotensin II, and its receptor are co-localized in inflammatory regions (2). All these results suggest that localized effects of activated RAS in the vascular wall can lead to infiltration of leukocytes into the vascular wall, which is a key step in atherosclerosis occurrence (5).

Although, angiotensin II may cause endothelial dysfunction by reducing access to NO. These findings indicate the role of angiotensin II and RAS in the treatment of atherosclerosis and cardiovascular disease (2).

### *Renin-angiotensin system*

Recently shreds of evidence have shown that the major risk factors for cardiovascular diseases can affect the RAS and increase the angiotensin II synthesis, norepinephrine secretion, endothelial dysfunction and neutrophil activation that can cause oxidative stress which leads to cardiovascular disorders (12).

### *Renin*

Renin is a pepsin-like aspartyl protease. Renin secretion is initially as a precursor molecule with the molecular weight of 55 kDa. Then it is broken during packaging in the Golgi apparatus and converted to reactive renin with the molecular weight of 44 kDa (13). Renin only affects its specific substrate called angiotensinogen, by breaking the bond between leucine 10 and valine 11 in N terminal. Renin produces angiotensin I.

### *Angiotensinogen*

Angiotensinogen is a alpha 2 globulin that is synthesized by the liver and is a renin substrate. It is glycosylated and weighs between 61 and 65 kDa. It may also be produced by non-liver tissues, including the brain and kidneys (14).

### *Angiotensin I, II*

Angiotensin I is made by the act of renin on angiotensinogen. Angiotensin I does not have any biological activity and rapidly converts into reactive angiotensin II by ACE (15).

### *Angiotensin-converting enzyme*

It is a zinc-dependent metalloproteinase that is widely

found in endothelial surfaces and epithelial cells (16). The enzyme is an exopeptidase and is widely found in different tissues. The highest levels of ACE in humans have been found in kidney, ileum, duodenum, and uterus. The lowest level of the enzyme is in the lungs, prostate, jejunum, testicles and adrenal glands.

In the RAS, ACE converts the inactive angiotensin I decapeptide into active angiotensin II octapeptide, which is the main active product of RAS (16).

### *ACE enzyme gene*

In humans, the ACE gene location is in the long arm of chromosome 17 (17q23). The length of the gene is 21 kb and includes 26 exons and 25 introns (17). This gene codes two different isoforms: somatic isoform (sACE) with the molecular weight of 170 kDa, that expresses in somatic tissues, and testicular isoform (tACE) or germinal isoform (gACE) with the molecular weight of 100 kDa which are expressed in embryonic cells and testicles (18). These two isoforms are produced from two different promoters. Somatic isoform transcription starts from a promoter located in the 5<sup>th</sup> region of exon 1 (Spro). As a result, all exons will be transcribed, so in the mature form of mRNA, there is a somatic isoform of exons 1 to 26. The germinal isoform is transcribed through a special internal promoter which is a 91 bp piece in intron 12. The germinal isoform mRNA consists of exons 13 to 26 (19).

### *Controlling ACE levels*

Although several studies have been conducted about ACE, there is not much information about the regulation of the ACE enzyme mRNA expression. ACE can be induced by corticosteroids in endothelial cells culture, alveolar macrophages, and monocytes (20). It also appears that hyperthyroidism can increase ACE levels in the bloodstream (21). Additionally, studies have shown that protein kinase-C and CAMP dependent mechanisms can increase ACE activity in endothelial cells. According to studies conducted so far, it seems that the ACE germinal form is hormonally controlled especially by FSH/LH hormones, human gonadotropin hormone and testosterone (22).

### *Genetic control of ACE levels*

The plasma levels of ACE are nearly identical when measured in a person several times while varying between different individuals. These observations indicate long-term control of the plasma levels of the enzyme, possibly with a genetic origin.

### *Role of the renin-angiotensin system in the body*

Renin-angiotensin system is considered as a hormonal system that controls blood pressure, blood flow, fluid volume, and electrolyte balance (23). RAS controls Long-term regulation of blood pressure and blood volume in the body. Renin is secreted by juxtaglomerular cells under

reduced volume conditions. This enzyme cuts the inactive peptide of angiotensinogen, which is synthesized by the liver and produces angiotensin I. Angiotensin I also converts to angiotensin II by ACE function (24).

Angiotensin II plays a key role in RAS and can cause narrowing of the vessels, through interaction with its specific receptor (25). Moreover, it releases aldosterone by affecting adrenal cortex. Aldosterone increases the sodium and water reabsorption from the urine by affecting renal tubules. These lead to an increase in the volume of blood fluids, compensate the reduced volume and increase blood pressure (17). Angiotensin II is also associated with cell growth and proliferation by inducing growth factors and cytokines (23).

### *Role of the renin-angiotensin system in cardiovascular diseases*

Renin-angiotensin system is considered as one of the main regulatory systems for cardiovascular physiology and has a role in cardiovascular system improvement, sodium homeostasis and maintaining vascular traction (11). It also plays a role in cell growth and damaged vascular walls repairment. Therefore, it is suggested that the ACE enzyme can be a candidate gene for influencing the cardiovascular disease process. An elevated level of ACE increases the conversion of angiotensin I to II (8).

### *Polymorphism*

In biology, polymorphism is the occurrence of different genotypes that cause different morphs or forms that can produce different proteins. These proteins might have different properties or different levels of functions (18).

### *Polymorphisms in the ACE gene*

Due to the important role of the RAS in the body, scientists have conducted extensive research to identify its polymorphisms and their possible role in the development of various diseases. Three polymorphisms, 4656(CT) 3/2 (also named 23945(CT) 2/3), 2350G/A (14521G/A, rs4343), and I/D (insertion/deletion, rs1799752) were among these discovered polymorphisms. Scientists have done extensive research, especially on I/D polymorphism. Among the various polymorphisms in the RAS, I/D polymorphism seems to have the most important relationship with the risk of cardiovascular disease.

### *Angiotensin-converting enzyme insertion/deletion polymorphism*

The first study on the ACE gene was carried out in 1988 by Cambien et al. They determined that the possible impact of a particular gene is responsible for 29%, 29% and 75% of ACE levels changing, respectively in fathers, mothers and children. Furthermore Regan et al found I/D polymorphism in 1990 (22).

This polymorphism includes presence (Insertion-I) or absence (Deletion-D) of a piece of 287 base pair in

intron 16. They observed that the average ACE activity in individuals with DD genotype was twice than genotype II. The enzyme activity in individuals with ID genotype was the intermediate activity observed in the genotypes mentioned (10). The ID polymorphism accounts for 47% of the observed changes in ACE levels. Also, the effects of this polymorphism are not limited to levels of ACE in the plasma and also affects ACE tissue levels (9).

A total of 160 polymorphisms have been identified for the ACE gene, so far that most of them are single-nucleotide polymorphisms. Only 34 of these polymorphisms are in the coding regions and 18 of them are silent mutations (17).

#### ***The association of ACE I/D polymorphism with cardiovascular disease***

ACE I/D polymorphism and its relationship to enzyme activity levels were first investigated in 1990 by Rigat et al (23). Although it seemed impossible to consider this polymorphism as a functional variant due to its location in the non-coding region, the study by Rigat et al, and the relationship between this polymorphism and the level of enzymatic activity were the starting point for various studies in this field (23).

The ACE gene polymorphism has been studied in a variety of studies. It has been specified that these polymorphisms, in addition to changes in blood pressure, are associated with several cardiovascular diseases including endothelial dysfunction, atherosclerosis, and heart failure (13). Studies have shown that DD and DI genotypes have been associated with a higher prevalence of hypertension and cardiovascular disease. The D allele is associated with elevated ACE levels, resulting in increasing the risk of cardiovascular disease by increasing angiotensin II and decreasing bradykinin levels (15).

Angiotensin II plays an important role in the development of atherosclerosis by increasing the release of growth factors derived from macrophages and platelets, inducing LDL oxidation and increasing the expression of chemokines, cytokines and adhesive molecules (3). ACE also disrupts endothelial cell function and promotes atherosclerosis by reducing nitric oxide secretion from the bradykinin-kallikrein system (3).

The association between ACE I/D polymorphism and cardiovascular disease has been challenged in various studies. Many of these studies have shown the association between polymorphism and disease risk, for example, in a large case-control study have confirmed an association between the risk of CAD and the D allele, and studies in the large population of Chinese showed that the DD genotype is involved as a risk factor in the development of primary atherosclerosis in the carotid arteries. In addition, various studies conducted in the middle population of Turkey, the white population of Western Australia, and the Caucasian population confirm the above results (15,21).

Numerous meta-analytic studies have been performed

in this field, most of which have shown an association between certain genotypes of polymorphisms and increased disease risk. To investigate the role of ACE I/D polymorphism in increasing the risk of cardiovascular disease in the population, a meta-analysis conducted by Zhou et al (24). In this study, 46 different studies including 5215 patients and 4782 healthy individuals were reviewed. They showed a significant association between DD genotype and the risk of cardiovascular disease (24).

However, studies in various populations have found no association between polymorphism and disease. In 2012, a meta-analysis conducted by Agerholm-Larsen et al to determine the association between ACE I/D polymorphism and angiotensin converting enzyme activity in plasma and their relation in blood pressure, risk of myocardial infarction, ischemic heart disease and ischemic cerebrovascular disease. They reviewed 46 different studies, including 32715 white people. The results showed that ACE I/D polymorphism could increase plasma enzyme activity but had no significant effect on blood pressure. In addition, although studies in small populations confirmed the association between this polymorphism and an increased risk of ischemic heart disease and ischemic cerebrovascular disease, studies in large populations confirmed no association between polymorphism genotype type and disease risk ACE I/D polymorphism and other diseases (25).

Due to the central role of ACE in the RAS, various studies have been conducted on the association of I/D polymorphism with micro vascular diseases, especially diabetes. Studies have shown that D alleles are significantly associated with an increased risk of diabetic nephropathy. In addition, studies have been done on the role of I/D polymorphism in Alzheimer's disease. Due to the role of ACE in the decomposition of the beta-myeloid peptide, increased levels of ACE appear to be protective against Alzheimer (15).

#### **Conclusion**

We reviewed 80 research and review articles relevant to this topic directly or indirectly. From the information given in these papers, the following aspects were drawn out. CAD and atherosclerosis are one of the main causes of mortality, especially in developing countries. In many of the population, polymorphisms play a role in the development of a disease by affection enzymes levels and activity. In some population, these effects are not observed. The results can be due to differences in ethnicity in different parts of the world. Most studies confirmed that ACE I/D polymorphism and DD genotype is involved in the progression of atherosclerosis. People with DD genotype have higher ACE activity and angiotensin II. Angiotensin II is an atherogenic factor that increases the production of adhesion molecules and foam cell formation in macrophages that lead to atherosclerosis. Therefore, DD genotype can increase the risk of disease. Conflicting

results from various studies related to the role of this polymorphism in the development of atherosclerosis could be attributed to the difference in ethnic background, gene-environment interaction, differences in the stage of disease and sample size.

In addition, the different results obtained teach us that considering only genetic factors or environmental factors cannot lead us to a deep understanding of diseases or give us the power to predict disease. Therefore, in addition to the need for further studies in this field, simultaneous consideration of environmental factors and people's lifestyles can be helpful in this direction.

#### Authors' contribution

NN was the principal investigators of the study. SP, MY and AAK were included in preparing the concept and design. NN and SP revisited the manuscript and critically evaluated the intellectual contents. All authors participated in preparing the final draft of the manuscript, revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

#### Conflicts of interest

The authors declare that they have no conflicts of interest.

#### Ethical issues

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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