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The biochemical fundamentals of angiotensin converting enzyme (ACE) gene polymorphism in myocardial infarction

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Abstract

Myocardial Infarction (MI) is the most important reason of mortality and morbidity in developed countries. It is detected that about half of the deaths, no matter what reason is, in the USA is sourced from MI. In recent years, a lot of scientific studies have increased on some genetic risk factors responsible from the formation of MI. One of the most popular of these is searching for the relations between the I/D polymorphism in the ACE gene and MI. In this study, it is assumed that ACE I/D gene polymorphism is a useful indicator in the detection of the risk of cardiovascular disease, the better control of the people in high risk group and the optimal cure to start at an earlier stage. By the help of these studies the risk factors of genetic origin, using the genetic indicators and new risk factors, and supporting the information by some easily performed biochemical experiments would supply great easiness in diagnosis and cure and save life.

Key words: Angiotensin converting enzyme, ACE gene polymorphism, renin-angiotensin system, myokard infarction

Miyokard infarktüsünde angiotensin dönüştürücü enzim (ADE) gen polimorfizminin biyokimyasal temelleri

Özet

Gelişmiş ülkelerde en başta gelen morbidite ve mortalite nedeni miyokard infarktüsü ve koroner arter hastalıklarıdır. Miyokard infarktüsü ABD'de ve diğer birçok gelişmiş ülkede tüm nedenlere bağlı ölümlerin yaklaşık yarısından sorumludur. Son zamanlarda miyokard infarktüsü oluşumundan sorumlu olabilecek bazı genetik risk faktörleri üzerinde çalışmalar artmıştır. Bunların arasında en popüler olanlarından biri Angiotensin Dönüştürücü Enzim (ADE) genindeki I/D polimorfizmi ve miyokard infarktüsünün ilişkisini araştıran çalışmalardır. Miyokard infarktüsü ve koroner arter hastalıklarına yol açan genetik belirteçlerin saptanması, bu hastalıkları önleme ve tedavi çalışmalarında kolaylık sağlayabilecektir. Bu genetik belirteçlerin önceden saptanması durumunda klinik belirtiler ve kardiyovasküler komplikasyonlar ortaya çıkmadan yüksek risk grubundaki kişiler belirlenebilecekler ve uygun tedavi için takibe alınabileceklerdir. Tüm bu çalışmalar ADE gen polimorfizminin kardiyovasküler riskin tahmininde, yüksek risk gruplarındaki hastalara erken ve etkili tedavinin planlanması ve önceden belirlenmesinde ışık tutabileceği düşüncesinden hareketle sürdürülmektedir.

Anahtar sözcükler: Angiotensin dönüştürücü enzim, ADE gen polimorfizmi, renin-angiotensin sistemi, miyokard infarktüsü

The biochemical fundamentals of angiotensin converting enzyme (ACE) gene polymorphism in Myocard Infarction (MI)

Cardiovascular diseases especially Myocard Infarction (MI) is the main cause of death in developed countries and their relation with certain risk factors like hypertension, cigarette smoking, obesity, male gender, hyperlipidemia and diabetes mellitus is well known. Its prevalence varies among different populations, epidemiological and familial studies have shown genetic and environmental factors cooperating in the pathogenesis of cardiovascular diseases.

MI is a multifactorial disease, influenced by environmental and genetic factors (Ortega et al., 2002). These factors differ in each population. Angiotensin Converting Enzyme (ACE) has an important impact on cardiovascular structure and function. ACE has a key role in the production of angiotensin-II and in the catabolism bradykinin, two peptides involved in the modulation of vascular tone and in the proliferation of smooth muscle cells.

Thus the ACE gene is a logical etiological candidate for MI. Several studies have suggested that the genes encoding components of the Renin-Angiotensin System (RAS) are candidate genes for cardiovascular disease and recently genetic polymorphisms of the RAS have been associated with cardiovascular diseases (Cambien et al.,1992; Jeunemaitre et al., 1997; Fatini et al., 2000).

Renin-Angiotensin System (RAS)

The Renin-Angiotensin System (RAS) plays a key role in regulation of arterial blood pressure and blood volume in normal individuals. RAS is one of the major regulators of blood pressure and fluid and electrolyte homeostasis. This is mediated through its constrictive actions on vascular smooth muscle and by its influence on aldosterone secretion from the adrenal cortex, electrolyte transport in kidney tubules and on thirst as well as sodium appetite in the brain (Peach et al., 1977; Reid et al., 1978).

Angiotensinogen, renin, angiotensin converting enzyme (ACE), angiotensin-II and angiotensin-II receptors are primary components of renin-angiotensin system. Genetic variants have been identified in several components of the RAS. The reported association of insertion/deletion polymorphism of the angiotensin converting enzyme gene with MI and coronary artery disease has thus generated continuing interest (Arbustini et al., 1995; Bohn et al., 1993; Cambien et al., 1992; Dzau et al., 1988; Friedl et al., 1995; Mattu et al.,1995). Polymorphic markers in angiotensinogen and angiotensin converting enzyme genes have been found to be associated with risk for MI. One of the polymorphisms is associated with risk for MI especially in subjects carrying the D allele of the ACE gene. Various studies considered four different genotypes:

1- The ACE insertion/deletion (I/D) polymorphim involving a 287-base pair (bp) Alu repeat sequence in intron 16 of the ACE gene
2- The methionine _ threonine variant at position 235 (M235T) in exon 2 of the AGT gene
3- The threonine _ methionine variant at position 174 (T174M) in exon 2 of the AGT gene and an A1166 _ C transversion in the 3' untranslated region of the AT1R gene (Ganong et al., 1995; Tiret et al., 1994).

The ACE gene contains a polymorphism based on the presence (insertion [I]) or absence (deletion [D]) within an intron of a 287-bp nonsense DNA domain, resulting in three genotypes (DD and II homozygotes, and ID heterozygotes (Lindhpainther et al., 1995).

The association between the ACE DD genotype and myocardial infarction, which was first described by Cambien and colleagues in the European subjects of the ECTIM study, has been confirmed in several populations of North American, Japanese, Italian, and Austrilian descent (Arbustini et al., 1995; Arca et al., 1998; Cambien et al., 1992; Ludwig et al., 1995). However several different studies did not show this association in populations from Austria, Finland, North America, Denmark, Japan, and New Zealand and a recent meta-analysis has indicated that besides ethnic difference and posible selection bias in most of these studies, a degree of bias towards positive results, at least in the smaller studies, seems likely (Arca et al., 1998; Agerholm-Larsen et al., 1997; Friedl et al., 1995; Katsuya et al., 1995; Lindpainther et al., 1993; Miettinen et al., 1999; Samani et al., 1996).

Renin

Molecular biological and biochemical measurements have opened a new era in our understanding of this important hormonal system (Murphy et al., 1991; Sasaki et al., 1991). The main source of rennin is the juxtaglomerular cells of the afferent arterioles of the kidney. Rennin is a glycoproteolytic enzyme that is responsible for the first step in the formation of angiotensin-II. In contrast the other proteases of this class, rennin is highly specific for its substrate, angiotensinogen, and is most active at neutral pH. Rennin is a single chain aspartyl protease of molecular weight 37-40 kd and pI 5.2-5.8. Renin's primary structure contains double domains; that is, the amino- and carboxyl- termini contain areas of similar sequence, forming a two-lobed structure surrounding the active site. This catalytic region contains two critical aspartic acid residues, one contributed by each half of the molecule (Blundell et al., 1983; Dzau and Pratt et al., 1986; Gomez et al., 1990). The human renin gene has shown that is encoded by a 12.5 kilobase (kb) DNA sequence at genomic analysis (Bockxmeer et al., 2000; Caldwell et al., 1976). Angiotensinogen molecule, in turn, is converted to angiotensin-II by angiotensin converting enzyme. Angiotensin-II then binds to its receptor to mediate many different cellular effects (Canavy et al., 2000; Philips et al.,1993; Tahmasebi et al., 1999).

Angiotensinogen (Agt)

Angiotensinogen is the major substrate for rennin. Angiotensinogen is the ultimate precursor of angiotensin-II. Structurally, it is a globular glycoprotein of molecular weight 55-65 kd and pI 4.3-4.9, depending on the degree of glycosylation (Hansson et al., 1999; Leung and Carlsson, 2001). The average carbohydrate content is 13-14%. The majority of the circulating angiotensinogen most likely derives from the liver, in particular, the pericentral zone of the liver lobules (Clauser et al., 1989; Doolittle, 1983; Morris et al., 1979). Analysis of human genomic DNA indicates that there is a single gene for angiotensinogen. The gene is composed of five exons and four introns and encompasses approximately 13 kb of genomic sequence (Clauser et al., 1989; Morris et al., 1979).

Angiotensin Converting Enzyme (ACE)

ACE is a dipeptidiyl carboxypeptidase that converts angiotensin-I to the potent vasoconstrictor angiotensin-II and inactivates the vasodilator bradykinin. ACE has a key component within the RAS, where it hydrolyzes angiotensin-I to generate angiotensin-II (vasoconstrictor) and the kallikrein-kinin system, where it inactivates bradykinin (vasodilator). ACE has been extensively characterized and purified from several sources, including serum, lung, seminal fluid, and plasma. The molecular weight ranges from 140-160 kd for the endothelial angiotensin converting enzyme to 90-100 kd for the testicular form, depending on the carbohydrate content of the molecule (Aldermann et al., 1991; Erdos et al., 1990; Packer et al., 1992; Peach et al., 1977). ACE appears to influence the cardiovascular system at many sites and in multiple ways (Erdos et al., 1990; Dzau et al., 1994). ACE has an important impact on cardiovascular structure and function. Among other actions, it catalyzes the conversion of angiotensin-I to angiotensin-II and the breakdown of bradykinin to kinin degradation products. Both angiotensin-II and bradykinin are powerful vasoactive molecules on the cardiovascular system. Therefore, with its pivotal role in two important cardiovascular hormonal regulatory systems, the renin-angiotensin system and the kallikrein-kinin system (Ehlers and Riordan, 1990; Erdos, 1990). The angiotensin converting enzyme (ACE) is an important part of the renin-angiotensin system. It acts upon the decapeptide angiotensin-I converting it into the octapeptide angiotensin-II, a potent vasoconstrictor. It also inactivates the vasodilator bradykinin. Both angiotensin-II and bradykinin can influence proliferation of smooth muscle cells. ACE catalyzes the conversion of angiotensin-I to angiotensin-II and the breakdown of bradykinin to kinin degradation products. Angiotensin-II and bradykinin are powerful vasoactive molecules with multiple acute and chronic effects on the cardiovascular system (Bunning and Riordan, 1987; Das et al., 1977; Ehlers and Riordan, 1983; Oparil, 1983; Soubrier and Corvol, 1990). For these reasons, the human ACE gene has been a preferred target in unraveling the molecular architecture of cardiovascular diseases. Angiotensin converting enzyme is unusual in that 26-30% of its dry weight is carbohydrate, in the form of fructose,

mannose, galactose, N-asetil –glucosamine, and sialic acid (Das et al., 1977). Angiotensin converting enzyme also is a member of the family of zinc metallopeptidases and contains a molar equivalent of zinc that functions is the hydrolytic step of the catalytic reactions (Bunning et al., 1983; Das et al., 1977; Ehlers and Riordan, 1983). Angiotensin converting enzyme is a key component within the RAS and ACE is a dipeptidyl carboxipeptidase that converts angiotensin-I to the potent vasoconstrictor angiotensin-II and inactivites the vasodilator bradykinin (Lindpainther et al., 1995; Mattu et al., 1995). ACE gene is polymorphic (Cambien et al., 1992). The ACE gene has been mapped to chromosome 17q23, and an insertion/deletion (I/D) polymorphism, involving a 287 base-pair alu repeat sequence, has been located to intron 16. (Cambien et al., 1992; Rigat et al., 1990; Samani et al., 1996; Soubrier et al., 1988, Tiret et al., 1993).

An insertion/deletion (I/D) polymorphism in the ACE gene results in genotypes II, ID, and DD (Lindpainther et al., 1995; Rigat et al., 1990). Whereas those people with DD genotype had the highest ACE plasma level, those with II genotype had the lowest. Since people with DD genotype and D allele have a higher level of angiotensin-II due to the higher level of ACE in circulation and tissues compared to other genotypes and alleles, DD genotype and D allele can be considered as a risk factor for a more severe cardiovascular damage (Cambien et al., 1992; Lindpainther et al., 1995; Samani et al., 1996; Tiret et al., 1993). ACE D allele is considered as the reason behind a higher ACE activity in both old and young populations (Frossard et al., 1998). According to the method of Lindpainther et.al. mistyping of I/D heterozygotes was controlled using insertion specific primers (Lindpainther et al., 1995). In 1992 in a retrospective, multicenter, case-control study, Cambien et al. reported that the frequency of the DD genotype was increased in subjects with MI recruited between 3 and 9 months after the event. Since then, studies both supporting the finding as well as those questioning the veracity of the association have been published (Cambien et al., 1992; Herbert et al., 1985; Soubrier et al., 1988). The findings in the meta-analysis are consistent with data linking the D allele to coronary artey disease risk using other criteria, particularly the findings in several studies of an increased familial risk of MI in those carrying the D allele (Badenhop et al., 1995; Beohar et al., 1995; Bohn et al., 1993; Evans et al., 1994; Ludwig et al., 1995; Mattu et al., 1995; Tiret et al., 1994). In a case control study of four different populations, a homozygous deletion allele in the gene for ACE was associated with an increased risk of MI, especially among individuals with below-average lipid and body mass (Cambien et al., 1992). The presence (allele I) or absence (allele D) of the 287 bp Alu repeat in intron 16 of the ACE gene was determined by evaluating the size of DNA fragments after polymerase chain reaction amplification, using the primers and PCR conditions described by Rigat et. al (Cambien et al., 1992; Mattei et al., 1989; Rigat et al., 1992; Soubrier et al.,1988). Because 4-5 % of samples with the I/D genotype were misclassified as DD with older methods, each sample found to have the DD genotype was subjected to a second PCR amplification with insertion-specific primers (5'TGGGACCACAG CGCCCGCACTAC3' and 5'TCGCCAGCCCTCC CATGCCCATAA3') with 67oC as the annealing temperature to avoid DD mistyping (Lindpainther et al., 1995). The ACE genotypes were assessed by PCR using primer sequences and PCR cycling conditions, as described previously. According to the absence or presence of the 287 base pair insertion in the PCR product, the patients were classified as homozygous DD or II, or heterozygous ID. To prevent mistyping of ID as DD genotypes, a second PCR with an insertion specific primer (5'TTTGAGACGGAGTCTCG CTC3') was performed in all samples classified as homozygous DD in the first PCR. Amplified DNA was electrophoresed in 2% agarose gels and visualised by ethidium bromide staining. Subjects with one 490 bp band on an agarose gel (2%) classified as II (insertion), subjects with both 490 and 190 bp bands were classified as ID, and subjects with only a 190 bp band were classified as DD. The molecular weight of ACE ranges from 140-160 kd for the endothelial ACE to 90-100 kd for the testicular form, depending on the carbohydrate content of the molecule (Ehlers and Riordan, 1983). Several biologic actions of ACE could be involved in the pathogenesis of coronary artery dsiease and myocardial infarction; the activation of angiotensin_I and the inactivation of bradykinin potentially result in decreased tissue perfusion, and angiotensin-mediated promotion of growth may be involved in the pathogenesis of cardiac diseases (Fabris et al., 1990; Jandeleit et al., 1991;

Lindpainther et al., 1993; Magrini et al., 1988; Mochizuki et al., 1992; Ridker et al., 1993). In a study undertaken by Eicher and his colleagues in USA on a patient group of 576 male and 124 females, the relationship between ACE DD genotype and MI was investigated. The gene frequencies were taken as a basis in the study and the most common genotype in the population was detected to be the ID genotype. DD and II genotypes were detected almost in the same frequencies. Among the patients with MI, individuals with an MI history in the family had significantly higher DD genotype (Eicher et al., 2001). Fatini and his colleagues claimed that ACE DD genotype is risk factor for MI patients based on a study on a sample taken from Italy (Fatini et al., 2000). A study by Marian et al. DD genotypewas associated with an excess of parental history of fatal MI. These results strengthened the hypothesis that genetic variation in the ACE gene may contribute to an increased risk for MI. Recently, an association of the D allele with cardiomyopathy and sudden death has been reported (Marian et al., 1993). In another recent study, 82 consecutive patients with known ACE genotype were followed up after percutaneous transluminal angioplasty. Restonosis was significantly more frequent among patients carrying the DD genotype than among those with ID or II genotypes (Bohn et al., 1993). In contrast to these studies, Fatini and his colleagues noted that they did not find a statistically significant relation between ACE I/D gene polymorphism and MI based on a study conducted with 304 MI patients in Canarian Islands, Spain (Ortega et al., 2002). The renin-angiotensin system is highly regulated and may contribute to the development of coronary artery disease and myocardial infarction by several mechanisms. The fact that ACE insertion/deletion gene polymorphism is not associated with coronary artery disease and myocardial infarction in large patient populations does not exclude interactions of this polymorphism in the renin-angiotensin system, which have been shown to increase the risk of coronary artery disease and myocardial infarction (Nakauchi et al., 1996; Tiret et al., 1994). The insertion/deletion I/D) polymorphism of the angiotensin-converting enzyme (ACE) gene was studied in patients with coronary heart disease (CHD) and healthy individuals randomly sampled from the Moscow population. The ACE gene proved to be associated with the plasma apolipoprotein B (ApoB) content in CHD patients, but not associated with HCD development in individuals with elevated serum cholesterol and triglycerides. An association was not revealed between the alleles of the ACE gene and hypertension in CHD patients (Shadrina et al., 2001). Recent evidence suggests that an insertion/deletion (I/D) polymorphism of the gene encoding angiotensin-converting enzyme (ACE) is associated with myocardial infarction and related cardiovascular diseases. We investigated a possible association of the ACE polymorphism with essential hypertension in a total of 263 cases/controls from among the elderly (age, over 70 years) and middle-aged (age between 30 and 60 years) Japanese population. The frequency of the I/I homozygote was significantly higher in hypertensive subjects than in controls in the elderly age group (33/57 vs 16/46; P = 0.02), but no association was observed in the middle-aged group (25/75 vs 26/85; P = 0.71). Similarly, having at least one insertion allele was associated with essential hypertension in the elderly age group (83/114 vs 46/92 in controls; P = 0.001), but not in the middle-aged group (78/150 vs 94/170; P = 0.524). These data suggest that genetic variation at the ACE locus may be associated with some determinants for blood pressure in elderly persons, and imply the involvement of the ACE insertion/deletion polymorphism in the etiology of age-related essential hypertension in the Japanese population (Yoshida et al., 2000). The aim of Covolo's study was to investigate whether ACE genotype is associated with HF by comparing cases and controls. The study sample consisted of 229 cases with HF due to coronary heart disease or idiopathic dilated cardiomyopathy and 230 controls recruited from the general population. The ACE I/D genotype was identified using a polymerase chain reaction assay. No evidence was found to support an association between ACE genotype and HF (Covolo et al., 2003). Heart failure (HF) is the final outcome of virtually all cardiovascular diseases and is a major and increasingly serious public health problem. The renin-angiotensin system plays an important role in the pathogenesis of cardiovascular disease. Insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE) has attracted significant attention; it has been extensively investigated in a spectrum of cardiovascular phenotypes because of its correlation with serum ACE activity. There is controversy regarding the association

of ACE I/D polymorphism with cardiovascular disease.

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