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GENES OF RENIN ANGIOTENSIN SYSTEM AND CORONARY HEART DISEASE

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ABSTRACT

Coronary constriction, proliferation of smooth muscle cells and arrhythmia are involved in the pathophysiology of coronary heart disease and its complications such as myocardial infarction and sudden death. All these effects are favoured by high angiotensin II levels. Angiotensin II is the main effector molecule of the renin angiotensin system and it acts through angiotensin II type receptors. Genetically determined differences in the expression of the components of this system could adversely affect angiotensin II concentration and subsequently heart. Consequently each component of this system represents a potential candidate in the etiology of cardiovascular disease. In this article we review the variation of the angiotensin I converting enzyme, angiotensin II type I receptor and angiotensinogen genes and their association with cardiovascular disease.

KEY WORDS: Coronary heart disease, Gene polymorphism, Renin angiotensin system.

The origin of the multifactorial atherosclerotic coronary heart disease (CHD) as of all disease lies in the duality, of inborn predisposition and the exposure to environmental influences. The contribution of tobacco usage, high blood pressure, dyslipidaemia, diabetes are all well known in aggravating the atherosclerotic lesion, precursor of which exists in the childhood. Family history of premature CHD is also a strong risk factor (1). Framingham database shows that the probability of a 50 year-old man with no known risk factors, having a coronary event in the next 10 years is 6%. In a 60 year-old man with the same cardiac risk profile, this probability increases to 9.0% within 10 years, suggesting the existence of other still unknown risk factors for atherosclerosis (2). Hence considerable research activity is directed on identifying other inherent risk factors that may have some genetic basis.

Given the complex pathophysiology of coronary atherosclerosis and the process that leads to the clinical symptoms there are number of candidate genes, which are studied. The very old and main target genes are those involved in lipoprotein metabolism such as apolipoproteins, lipolytic enzymes and lipoprotein receptors. Other genes are those of thrombotic factors, endothelial dysfunction, plaque formation and plaque rupture. Recently newer risk factors such as apolipoprotein E (apo E), homocysteine, lipoprotein(a) (Lp[a]) and genes of renin angiotensin system (RAS) are also included. In Indian population our laboratory has already studied the association of genes of low density lipoprotein (LDL) receptor (3) apo E and serum levels of Lp(a) (4), methylene tetrahydrofolate reductase (MTHFR) (5), with CHD. In this article we review the genes of RAS and their association with CHD.

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Renin Angiotensin System

Renin angiotensin system (RAS) has an important role in the pathogenesis of atherosclerosis. This enzyme cascade is one of the important factors regulating blood pressure and fluid

and electrolyte balance (6) and it also influences cardiovascular structure (7). Two distinct functionally similar and perhaps interrelated renin angiotensin systems have been identified. The initiating enzyme renin is cleaved from prorenin in several tissues but mainly in the juxtaglomerular apparatus of the nephrons. Its substrate angiotensinogen (AGT), an α-globulin is found both locally and systemically. Renin cleaves AGT to an inactive decapeptide, angiotensin I that is further proteolysed mainly by tissue bound angiotensin I converting enzyme (ACE) and serine proteinases (8) to an active octapeptide angiotensin II (AII); which has growth promoting and vasoconstrictive effects mediating through angiotensin II type 1 receptor (AT1R). [Fig. 1].

The hallmark of the CHD is an atheroma, a fibrous fatty plaque in the subintima that consist of a center, that has varying proportion of intracellular and extracellular lipids, altered macrophages and T- lymphocytes and is surrounded by fibrous connective tissue, myoepithelial cells and proliferating smooth muscle cells (1). Angiotensin II plays an important role in various component pathways involved in atherosclerosis. It stimulates various growth factors, promotes smooth muscle hypertrophy and hyperplasia by cytosolic and intranuclear mechanisms. Angiotensin II oxidizes the LDL cholesterol particle thereby promoting the uptake into the endothelium. It also causes endothelial dysfunction wherein the blood vessels

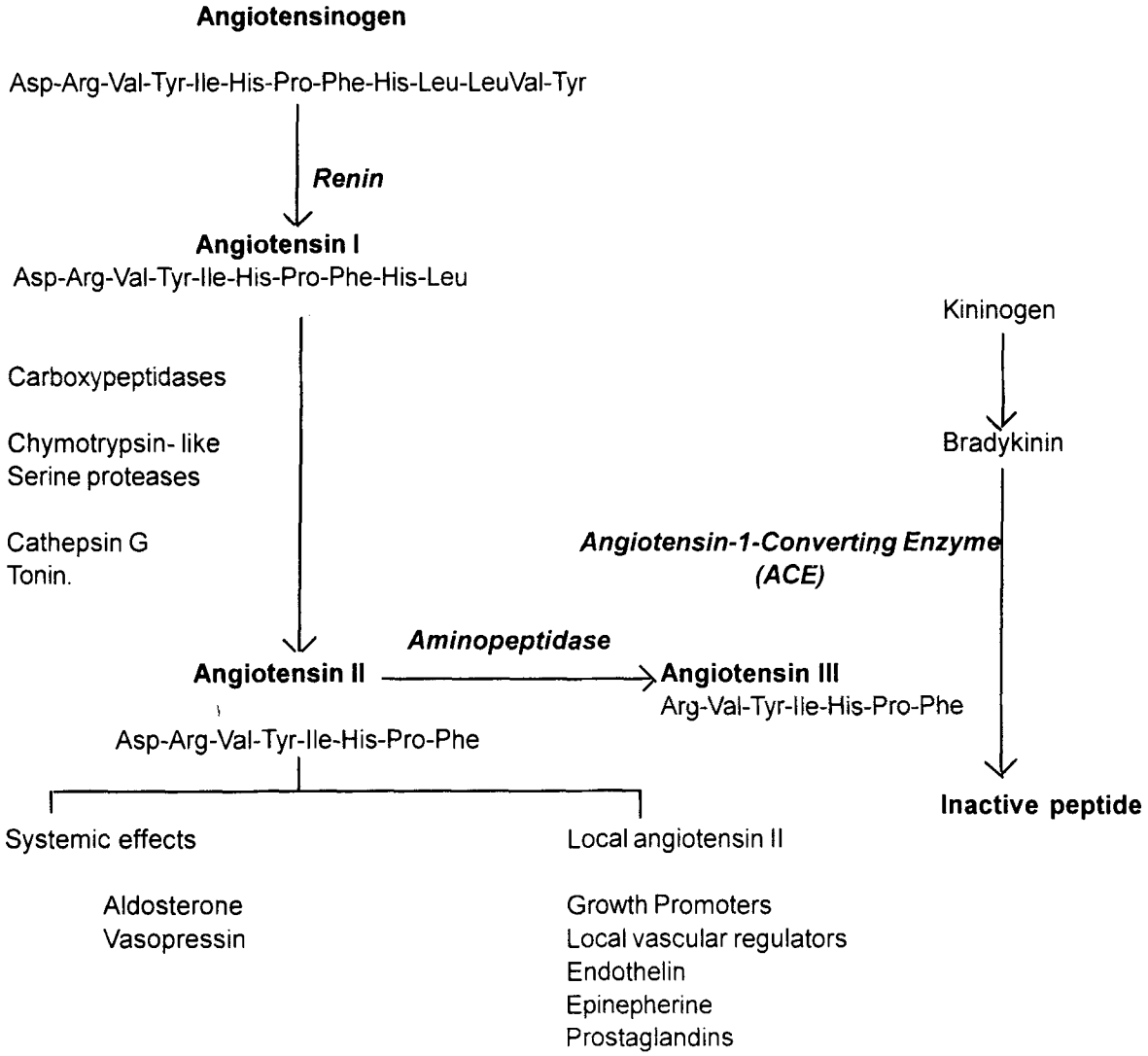

Fig. 1: Renin Angiotensin System

have angiographic narrowing. AII may also be an important factor in mediating plaque instability and blocks natural fibrinolytic pathway. It is apparent that AII is not only involved in the development of atherosclerosis but it also possibly predisposes early ischemic events by more than one mechanism and at more than one site (9). Allelic variations in the components of this system may affect AII concentration and thus have led to the investigation of the association of the genetic variation of ACE, AT1R and AGT with CHD.

Angiotensin I Converting Enzyme (ACE) {EC-3.4.15.1}

Because the generation of AII depends on ACE it is quite conceivable that populations with enhanced expression of ACE would have higher incidence of coronary events than other subpopulations. ACE, a carboxypeptidase; exists predominantly as an ectoenzyme of vascular endothelial cells and is also a component of the Kallilrein kinin systems where it inactivates bradykinin (BK); a potent vasodilator.

Plasma ACE levels are stable with time within individuals but show marked inter-individual variability, 50% of which is accounted for by a major gene effect (10). The human ACE gene contains 26 exons and is mapped to chromosome 17q23. After its cloning, the ACE gene was shown to be characterized by an insertion / deletion polymorphism based on the presence (insertion [I]) or absence (deletion [D]) within intron 16 of 287 base-pair alu repeat sequence, resulting in three genotypes D/D and I/I homozygotes and I/D heterozygote (11). The I/D polymorphism was found to be in strong linkage disequilibrium with the major gene locus controlling plasma ACE with mean plasma ACE level in D/D subjects approximately twice that of II subjects, with I/D subjects having intermediate values (12,13). With this established fact various studies have been carried out to show an association of D allele with CHD in various different populations.

Ruiz et. al. (14) studied the association between ACE gene polymorphism and atherosclerosis in subjects with both insulin and non-insulin dependent diabetes mellitus. They found that subjects with atherosclerosis had a higher frequency of the DD genotype compared with controls in both groups. A progressively increase relative risk in individuals with heterozygous and homozygous for the D allele was observed (Odds ratio- 1.41 and 2.35 respectively $p<0.007$) suggesting a co-dominant effect on the cardiovascular risk. In a large case-control study, Cambien et al (15), found that in 1300 subjects, the D/D genotype of ACE was associated with increased risk of myocardial infarction (MI). The subjects who took part in this ECTIM (Etude Cas-Temoin de P Infarct du Myocardae) study were recruited between 1989-1991 from the registers of the WHO/ Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) in Belfast (Northern Ireland), Lille (Northern France), Strasbourg (Eastern France), and Toulouse (Southern France). The DD genotype was found in 32.0% of patients and 27.3% of controls and was associated with 34.0% excess risk (adjusted odds ratio 1.34) for acute myocardial infarction (AMI), when compared with that for the patients with other genotypes, I/I and I/D. The association was significant in the so-called low-risk group consisting of subjects having the least conventional risk factors, such as hyperlipidaemia and hypertension. Tiret et al (16) studied ECTIM population and determined that in subjects with a DD genotype, the chance of having parents with a history of MI was increased three folds. The results of the Cambien study (ECTIM) were opposed by Bohn et al (17), Lindpaintner et al (18), Sanderson et al (19). A meta-analysis of 15 studies carried out between 1991 to 1996; have been reported by Samani et al (20). They calculated the overall odds ratio for CHD in patients with the ACE D/D genotype to be 1.26 compared with patients with ACE I/D and I/I genotype (95% CI-1.15-1.39, $p<0.0001$). In Indian population, a study carried out in our laboratory, by Joseph et al (21) demonstrated that the D allele of the ACE gene conferred no appreciable increase in the risk of CHD or MI. The D/D, I/D and I/I genotypes were found 20.66%, 46.66% and 32.66% of the patients and 23.38%, 49.75% and 26.86% of controls respectively. The reason for the negative association in this study could be ethnic heterogeneity and disease status

heterogeneity among the patients and controls. The serum ACE level in our population was according to ACE genotypes i.e. highest in D/D polymorphs, intermediate I/D and lowest in I/I genotypes. There was no significant difference in serum ACE levels between patients and controls. On similar grounds we have also studied ACE gene polymorphism in subjects suffering only from hypertension and have found no association (22,23). However D allele was significantly associated with an early onset of hypertension and although non-significant the D allele frequency was highest in subjects with family history of CHD (23). Patients with primary hypercholesterolaemia are known to be at substantially increased risk for the premature development of CHD. O' Malley et al (24) have shown the association of D/D genotype of ACE and hypercholesterolaemia (40% D/D versus 16% I/D + I/I genotypes respectively; $p<0.02$) and suggested that ACE genotypes in asymptomatic familial hypercholesterolaemia and familial defective apolipoprotein B-100 provide additional means to identify patients at greater risk for the development of CHD.

Angiotensin II type I Receptor (AT1R)

Following the discovery of a functional variation in the ACE gene research has identified another genetic variation in RAS that may contribute to cardiovascular disease i.e. in angiotensin II type I receptor (AT1R). Two subtypes of angiotensin receptors have been identified; AT1 and AT2 using ligand binding studies. The AT1R, through which are exerted most of the actions of AII, is a G protein coupled, spanning seven transmembrane domains (25). The human AT1R gene locus is mapped to chromosome 3q21 and 25 (26). The gene is large (47kb) and contains 5 exons. The single coding exon with the AT1 open reading frame (ORF) is found on exon 5. In the AT1 receptor gene five polymorphisms have been identified. However only A/C transversion at nucleotide position 1166 in the 3' un-translated region of the AT1R gene was found to be associated with human essential hypertension as reported by Bonnardeaux et al (27). A significant increase in the allele frequency of C 1166 in hypertensive subjects was seen (0.36 versus 0.28 in normotensive subjects; $x^2 = 6.8$, $p<0.01$). In contrast to the deletion polymorphism in the ACE gene, which is associated with the serum ACE activity, no clear phenotype has been determined in conjunction with the A/C genotype. Despite this, it is postulated that the AT1 receptor polymorphism may be associated with functional alterations in the responsiveness of this receptor. Some authors (28-30) confirmed these results of Bonnardeaux et al (27) but others did not (31-33). Benetos et al (33) did not show association of this polymorphism with essential hypertension; but reported an association with aortic stiffness and with ratio of total to high-density lipoprotein cholesterol. The possible interaction between lipids and expression of the AT1 receptor gene was also confirmed by Nickenig et al (34) who have reported an up-regulation of vascular AT1 receptor gene expression by LDL cholesterol in vascular SMC. This suggests that LDL cholesterol can modify receptor expression and thereby alter the renin angiotensin system status especially in persons with the AT1R AC/CC genotype. The A/C 1166 polymorphism was also shown to be associated with coronary vasoconstriction by Amant et al (35) and support the theory that the AT1 receptor polymorphism is involved in localized segmental hyper-reactivity in infarct-related vessels. Similar results are also documented by van Geel et al (36). In ECTIM study population, Tiret et al (37) found a significant interaction between ACE I/D polymorphism and the AT1 A/C polymorphism. The odds ratio for MI associated with ACE D/D genotype was found to be four fold higher in AT1 CC; homozygotes than AA homozygotes. They suggested that since the AT1R gene polymorphism does not seem to be independently and directly associated with MI, but modulates the relative risk conferred by the ACE D allele, it is associated with increased response of the receptor to AII. Functional G protein coupled receptors show desensitization after exposure to their agonists (38). This phenomenon can result from various mechanisms including receptor protein modifications and down regulation of gene expression (38). As no polymorphism was detected that modified the encoded amino acid sequence (27), they further speculated that the AT1R C allele is in linkage

disequilibrium with a functional variant that could alter the down regulation of AT1R gene in response to AII, which is present in increased concentration with ACE DD genotype. Similar kind of studies carried out, where the synergistic interaction between ACE D/D and AT1R A/C on MI was reported (39) while it was disapproved by others (40). AT1R C allele was also shown to be independently associated with CHD (41-43). An interesting study was carried out by van Geel et al (44) on TGR (AMHC-h AT1R) rat model, which over-expresses the human AT1 receptor in the myocardium, to study some of the associations between an increased AT1 receptor number and cardiovascular disorders. Their study indicated that the AT1 receptor polymorphism that may increase receptor number or sensitivity may not cause CVD directly but it can contribute to the process that is started by other factors such as high renin conditions. Although the above findings suggest a positive association between the AT1-receptor CC polymorphism and certain CVD, negative associations have also been reported in coronary atherosclerosis (45) and left ventricular hypertrophy (46). Recently Hilger et al (47) reported that A/C 1166 polymorphism of AT1R does not affect high BP, renal hemodynamic and aldosterone response to infused AII. In Indian population, we have studied the association of AT1R genotypes with CHD (48). We observed that there was no difference between the AT1R genotypes in patients and controls and no significant synergistic contribution of ACE D/D and AT1R A/C polymorphism to the increase risk of coronary artery disease. In hypertension group also similar results are observed. C allele of AT1R did not correlate with the age of onset of hypertension and the frequency was found low in subjects with family history (23).

Angiotensinogen (AGT)

Research has identified polymorphism in another important component of RAS, angiotensinogen (AGT); the earlier precursor of angiotensin II. The variants of which are reported to be associated with human essential hypertension. Plasma AGT is primarily synthesized in the liver under the positive control of estrogens, glucocorticoids, thyroid hormone and angiotensin II and is secreted through the constitutive pathway (49). Several observations point to direct relationship between AGT and BP; more specifically with plasma AGT levels (50) wherein plasma AGT levels differed between hypertensives and normotensives. The angiotensinogen gene has been mapped on chromosome 1q4 (51) and implicated in essential hypertension through both genetic linkage and allelic association by Jeunemaitre et al (52) initially in both Utah and French Caucasian and was confirmed later in two other sets of Caucasians hypertensive families by Caufield et al (53). Apart form rare mutations that potentially affect the kinetics of AGT generation (54) or the secondary structure and secretion of the protein (55), a missense muation in exon 2 of the AGT gene ($T^{704}\longrightarrow C$) encoding threonine instead of methionine at residue 235 of the mature AGT protein; was significantly more common in hypertensive subjects than in normotensive controls with or without T174M ($C^{521}\rightarrow T$) variant (0.14 and 0.33, n=215 than in controls (0.09 and 0.28, n=232); both differences were statistically significant [x^2= 5.6, $p<0.02$, x^2= 13.5, $p<0.01$] (52). Two other studies found an association with the T174M polymorphism, which is in complete linkage disequilibrium with M235T polymorphism (56,57). A variety of observations support the hypothesis that the causative mutation should affect the synthesis, function, secretion, or metabolism of AGT itself. Some reports have shown that the presence of the T235 allele has been associated with a 10% - 20% increase in plasma AGT level (52). Results obtained by the inactivation or duplication of the AGT gene in transgenic mice, by Kim et al (58) also showed a relationship between AGT gene expression, plasma AGT level and BP. Few reports have studied the association of M235T with coronary artery disease in Caucasians (45,57,59) and Japanese (60,61) individuals. Jeunemaitre et al (45) have shown significant correlation between the presence of AGT 235 T allele and the extent of the coronary lesions. ($r=0.19, P=0.04$) in patients with low risk status. In this study however, no significant association was found between ACE, AT1R and AGT gene polymorphisms and the clinical characteristics of MI and non-MI. Tiret et al's (57) study in the ECTIM population, have not shown any significant impact of AGT locus on non-fatal MI but have suggested it

to be involved in the predisposition to high blood pressure only in non-overweight men. In a case control study of a white population from New-Zealand, Katsuya et al (59) examined the M235T mutation and have reported that T235 homozygotes were at significantly increased risk of CHD (Odds ratio- 1.7, 2P=0.008) and of MI specifically (Odds ratio-1.8, 2P=0.009). The authors concluded that the T235 polymorphism of AGT gene is an independent risk factor that carries an approximately 2.0 fold increase risk of CHD. Among the Japanese studies carried out, Kamitani et. al. (60) and Ishigami et al (61) have reported positive association of M235T of AGT with CHD. Since the incidence of hypertension among both the patients and the control subjects was similar in Ishigami et. al. study (61), they suggested that M235T genotype of AGT gene is an independent risk factor for CHD. Recently Winkelmann et. al. (62) have reported significant relation between the AGT M235T variant, its protein product and cardiovascular phenotype and provided evidence for a possible role of elevated circulating AGT in the pathogenesis of CAD.

Certain observations regarding positive association of these gene polymorphisms and cardiovascular diseases suggest these as distinct heritable risk factors for cardiovascular morbidity. On the other hand certain negative associations raised doubts about the feasibility of using them as markers of CHD.

The knowledge of the physiologic features and clinical manifestations of RAS and evidence from several animal and human studies strongly support its involvement in the pathogenesis of CVD. Multiple reasons may explain why association studies of component genes of this system provide conflicting data: the low risk conferred by the RAS genotypes that have been identified so far, ethnic diversity, the often retrospective cross-sectional nature of the analysis, the low prevalence of the polymorphism and variations in population stability.

Further studies confirming the component gene variation in the pathophysiologic characteristic of CVD would constitute a land-mark discovery that would enable clinicians to stratify patients into various risk categories according to their genotypes and institute appropriate primary risk factor modulations. Although at present there is no compelling reason to target the molecular variants for treatment but the recognition of them as a predictor of risk is of particular interest. Further it may link to the existing target specific pharmacological agents present already in the form of receptor blockers and ACE inhibitors.

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