Renin-Angiotensin System Gene Polymorphisms and Hypertension

Priyanka Shankarishan¹, Prasanta Kumar Borah², Jagadish Mahanta ³

Abstract

Hypertension has emerged as a major public health problem in developing countries including India. Hypertension, a major cardiovascular risk factor is recognized as a multi-factorial trait resulting from the interaction of various environmental and genetic factors. The genetic contribution is speculated to make up about 30% to 40% of the variation in blood pressure. Identification of variant genes that contribute to the development of hypertension is further complicated because the cardiac output and peripheral resistance, are controlled by other intermediary phenotypes. Sodium has been postulated as the major intermediary in blood pressure regulation. Therefore, polymorphisms of candidate genes encoding proteins influencing renal tubular sodium transport, either directly or indirectly through effects on intra-renal hemodynamics, have been associated with differences in blood pressure level. Considering the importance of genetics on hypertension and the diversity of the related genes, evaluation of these genes and the study of new genes are necessary. It is hoped that by deducting related genes for essential hypertension in individual, will help in prevention of potential patients. We will be able to diagnose those at risk and develop new treatments for these patients.

Introduction

Hypertension has emerged as a major public health problem in India and in many developing countries. It is reported to be the fourth contributor to premature death in developed countries and the seventh in developing countries.¹ Hypertension, a major cardiovascular risk factor is recognized as a multi-factorial trait resulting from the interaction of various environmental and genetic factors. The genetic contribution is speculated to make up about 30% to 40% of the variation in blood pressure.²

Identification of variant genes that contribute to the development of hypertension is complicated by the fact that the two entities that determine blood pressure, namely cardiac output and peripheral resistance, are controlled by other intermediary phenotypes, including the autonomic nervous system, vasopressor/vasodepressor hormones, the structure of the cardiovascular system, body fluid volume, renal function and many others. Identification of genes controlling the variations in blood pressure, can define primary physiological mechanisms causing this trait, thereby clarifying disease pathogenesis, establishing molecular diagnostics and developing a novel mechanism to prevent premature death of people at risk of developing hypertension and perhaps new therapy for hypertension.

No single genetic variant has emerged from linkage or association analysis as consistently related to blood pressure level or definitive risk category (i.e. Hypertensive versus normotensive) in every sample and in all population. However, polymorphisms in candidate genes encoding proteins known to influence renal tubular sodium transport, either directly or indirectly through effects on intra-renal hemodynamics, have been associated with differential blood pressure.
Hypertension and the Renin – Angiotensin System

The Renin angiotensin system (RAS) is a coordinated hormonal cascade in the control of cardiovascular, renal, and adrenal function that governs fluid and electrolyte balance and arterial pressure (Figure 1).1

Through its modulation of salt and water homeostasis, the renin-angiotensin system (RAS) is a major regulator of blood pressure.2 Since role of RAS is involved in BP regulation, the genes that encode the components of RAS have been suggested to be promising candidate for understanding the pathophysiology of hypertension. Particularly, polymorphisms in genes of the renin-angiotensin-aldoctosterone system namely angiotensinogen, angiotensin-converting enzyme (ACE), angiotensin II type I receptor and aldosterone synthase, have been prominent. The end product of the renin-angiotensin cascade, angiotensin II, enhances renal tubular sodium re-absorption both directly and indirectly via stimulation of aldosterone synthase and release. Polymorphisms in two of these genes, namely angiotensinogen and ACE, have been associated with functional differences in the encoded gene products with measures of “sodium sensitivity”, as measured by blood pressure response to changes in sodium intake.

Genes of the Renin – Angiotensin System

Angiotensinogen

Given the strong correlation of plasma angiotensinogen levels and blood pressure, angiotensinogen (AGT) is one of the frequently studied genes which is located on 1q42 to 43, and comprises of five exons and four introns, spanning 13 kb.3 Angiotensinogen is a key protein in the renin–angiotensin system, which influences vascular tone, renal sodium reabsorption, and blood pressure.4

The cleavage of angiotensinogen by renin is the rate-limiting step in the cascade of enzymatic events leading to the generation of angiotensin. Angiotensinogen is also synthesized and present in many tissues in addition to the liver, such as the brain, large arteries, kidneys, and adipocytes.5 Therefore, modest changes in plasma or tissue angiotensinogen could play a major role in the generation of angiotensin I (Ang I) and in controlling blood pressure.

Angiotensinogen is a moderately abundant 55,000–60,000 Da serum glycoprotein that is the precursor to the angiotensin peptides and is the only known naturally occurring renin substrate. It is synthesized by a variety of cells, most prominently hepatocytes, adipocytes, and astrocytes. Most angiotensinogen is extracellular and is constitutively secreted. Thus, there is apparently no way that an organism can orchestrate rapid changes in angiotensinogen concentration. The angiotensinogen gene is regulated by several hormones (e.g., glucocorticoid and estrogen), and is an acute-phase protein. Angiotensinogen is a member of the serpin gene superfamily, but there is little reason to suppose that this protein is a serine protease inhibitor.6

Plasma levels of angiotensinogen (AGT) have received particular attention for several reasons.7 First, plasma AGT levels and diastolic blood pressures are correlated in some patients,8 and associations of AGT levels and hypertension have been demonstrated in families.9,10 Second, infusion of AGT into sodium-depleted but not sodium repleted rats raises their blood pressures,11 while administration of antibodies against AGT lowers pressure.12 Third, transgenic mice expressing high levels of rat AGT have elevated blood pressures.13,14 Fourth, human plasma AGT levels are near the Km of renin,15 so that variations in AGT levels will affect the rate at which renin converts AGT to angiotensin I.

Angiotensinogen (AGT) plays an important role in hydro-mineral balance and the control of blood pressure.16,17 Walker et al., (1979)18 observed a highly significant correlation between plasma AGT concentration and blood pressure in a large cross-sectional epidemiological study. Within the context of a family study, Watt et al., (1992)19 reported higher plasma AGT levels in young adults with an elevated blood pressure whose parents also had high blood pressure compared with young adults with low blood pressure whose parents also had low blood pressure. Plasma AGT is also reported to be higher in hypertensive subjects and in the offspring of hypertensive parents.20 In addition, over-expression of the AGT gene causes elevated blood pressure in transgenic mice carrying the rat AGT gene.21

Several reports show that the AGT genotype has a moderate but significant effect on plasma AGT concentration. Plasma AGT is elevated 20% in men and women carrying the 235T allele.22 Bloem et al., (1995)23 also found that the plasma AGT concentrations of normotensive white American children carrying the 235TT genotype were 13% higher than those with the 235 MM genotype. The mean plasma AGT concentration in African American children was 19% higher than in whites, but the association was not detected because the frequency of M235 was too low to show an association. Indeed, when splitting the 235T allele with another AGT gene polymorphism in African Americans, Bloem et al., (1997)24 found a significant association with plasma AGT. Busjahn et al., (1997)25 reported a trend to a co-dominant effect of the 235T AGT allele on plasma AGT concentration in twins, although the variation in the concentration was too great to reach significance. Another study on a large sample of the MONICA Augsburg cohort also found a co-dominant and significant increase in plasma AGT concentration in patients bearing the M235T variant.26

The AGT genotype could influence the amounts of AGT mRNA and AGT in tissues. No report has shown a relationship between AGT 235T genotype and tissue AGT protein concentration, but Morgan et al., (1997)27 described increased AGT 235T mRNA in the uterine spiral arteries of heterozygous women. These results offered a plausible explanation for the association and linkage of the AGT locus with the occurrence of pregnancy-induced hypertension.

Although several polymorphisms in the AGT region have been identified much interest has focused on two coding region polymorphisms, M235T and T174M, both in exon 2. More specifically, through case-control studies, many28,29 but not all30,31 investigators have concluded that the threonine allele (T235) of M235T and the methionine allele (M174) of T174M are associated with elevated risk for hypertension. The M235T mutation changes a nonpolar amino acid to a polar amino acid and thus potentially changes the tertiary structure of the
protein. Because of this, it is likely that such a mutation may also influence protein function.

A collaborative investigation of the AGT gene in siblings from Utah and Paris sharpened these findings by reporting both linkage and association of AGT molecular variants (235T and 174M) with hypertension, suggesting that these AGT polymorphisms may represent markers of an inherited predisposition to essential hypertension in humans. These findings have recently been extended to a Japanese population, where a significant association was also noted between hypertension and the 235T allele, along with a substantial increase in the population frequency of the 235T allele. The probable involvement of the AGT genomic region with blood pressure regulation is strengthened by two reports of an association between proteinuric preeclampsia and AGT polymorphisms: one with the 235T allele, the other with a microsatellite polymorphism.

Several subsequent linkage studies showed a relationship between the AGT locus and high blood pressure. In the United Kingdom, Caulfield et al., (1994) showed a strong linkage and association of the AGT gene locus with essential hypertension in a group of 63 white British families, despite the fact that there was no association between hypertension and the 235T variant. A weak linkage was found in 180 hypertensive Mexican Americans patients belonging to 46 large families living in the San Antonio, Texas area. However, the AGT locus showed no evidence of linkage to hypertension in a large multi-centric European study involving 630 affected sib pairs, even when patients with severe hypertension were selected. The implication of this gene in essential hypertension in Chinese people has also been challenged; there was no evidence of linkage in 310 hypertensive sib pairs from central China.

The common 235T variant does not affect the Km of renin, and its secretion and metabolism are similar to that of 235M AGT. However, the replacement of a methionine by a threonine residue is not neutral. Cohen et al., (1996) showed that 235M and 235T AGT could be readily distinguished by a set of monoclonal antibodies, which allows plasma immuno-genotyping of homozygous or heterozygous patients. Because the 235T variant is not functional per se, it could be a marker for a putative, as yet unknown, functional molecular variant that increases plasma AGT and mediates predisposition to hypertension.

**Angiotensin-converting Enzyme**

Angiotensin-converting enzyme (ACE) is a zinc metallopeptidase widely distributed on the surface of endothelial and epithelial cells. Angiotensinogen is converted to angiotensin I by stimulation of renin. ACE then converts angiotensin I to angiotensin II, the main active product of the renin–angiotensin–aldosterone system (RAAS). The human ACE gene is located on chromosome 17q23, and includes 26 exons. The coding sequence codes for a 1306 amino acid protein, including a single peptide. The gene product, ACE, is composed of 2 homologous domains with 2 active sites. The ACE gene product plays an important role in cardiovascular homeostasis as ACE converts angiotensin I to angiotensin II, and is involved in the degradation of bradykinin. Bradykinin acts as a potent stimulator of nitric oxide (NO) release. NO plays a crucial role in protecting the endothelium from injury. Furthermore, it has been reported that hypertensive effects are mediated in a bradykinin-dependent manner. These two actions make ACE inhibition a goal in the treatment of conditions such as high blood pressure, heart failure, diabetic nephropathy, and type 2 diabetes mellitus. Inhibition of ACE (by ACE inhibitors) results in the decreased formation of angiotensin II and decreased metabolism of bradykinin, leading to systematic dilation of the arteries and veins and a decrease in arterial blood pressure. In addition, inhibiting angiotensin II formation diminishes angiotensin II-mediated aldosterone secretion from the adrenal cortex, leading to a decrease in water and sodium reabsorption and a reduction in extracellular volume.

The ACE gene encodes 2 isoforms, namely, the somatic form (sACE), with a molecular mass of 170 kDa which is expressed in somatic tissues mainly in the lung, including vascular endothelial cells and epithelial kidney cells and the germinal form or the testicular form (tACE) with a molecular mass of 100 kDa is expressed in germinal cells in the testis.

The ACE gene, first described by Rigat et al., (1990) has an insertion/deletion (I/D) polymorphism in intron 16. The group published an important report that provided the impetus to further study polymorphisms in this gene. They found a polymorphism involving the presence (insertion, I) or absence (deletion, D) of a 287-bp sequence of DNA in intron 16 of the gene. Mean ACE activity levels in DD carriers were approximately twice that found in II genotype individuals. Subjects with the ID genotype had intermediate levels indicating codominancy. The I/D polymorphism accounted for approximately half (47%) of the observed variance in ACE levels in this study group. Later studies showed that the involvement of the I/D polymorphism is not limited to ACE levels in plasma, and is also detected in tissue ACE levels. The ACE I/D polymorphism were initially detected by restriction fragment length polymorphism (RFLP) analysis. The first polymerase chain reaction (PCR)-based detection of this polymorphism was reported by Rigat et al., (1992) who used a set of primers flanking the insertion sequence. Family based studies performed by Shanmugam et al., (1993) however, showed the possibility of mistyping ID heterozygotes with this PCR method. Preferential amplification of the shorter D allele may cause the misclassification of approximately 4 to 5% of ID genotypes to DD. An additional PCR amplification reaction was, therefore, formulated for the confirmation of DD genotypes obtained in the first standard PCR, including a new sense primer that is insertion-specific.

Various published reports suggest an association or linkage of the D allele of the ACE gene with myocardial infarction (Cambien et al., 1992), essential hypertension, left ventricular hypertrophy, renal insufficiency and high fasting blood sugar levels. However, some other investigators have found no association between ACE I/D polymorphism and hypertension.

Inter-ethnic variations in the frequency of allelic forms of certain genes have been suggested as one of the reasons for such discrepancies. This is particularly true for the ACE gene, since wide inter-ethnic allelic variations have been
Several other studies have shown that the DD genotype of the I/D polymorphism in the ACE gene is associated with hypertension and other cardiovascular risk factors. Significant association of hypertension with D allele of the ACE gene has been documented in the African-American, Chinese, and Japanese populations. However, many other studies have failed to detect any such association. Genetic and environmental heterogeneity among different ethnic groups may account for this inconsistent result.

In a linkage study, Jeunemaitre et al. (1992) found no evidence to support linkage between the ACE locus and essential hypertension. Likewise, in the Dutch Hypertension and Offspring Study, Schmidt et al. (1993) failed to find a significant association between the I/D polymorphism and blood pressure status in subjects with high or low blood pressure and in their offspring. This lack of association was repeatedly found in later studies. Several other studies, however, reported a positive association between the D allele and high blood pressure.

**Angiotensin II Type I Receptor**

The renin-angiotensin system comprises a cascade of enzymatic reactions, which results in the production of angiotensin II from the angiotensinogen substrate. The physiological effects of angiotensin II are mediated by a final common pathway, through angiotensin II receptors on the cell membrane. Two isoforms of endothelial receptors for angiotensin II are known so far: AT1 and AT2 using ligand binding studies. Most of their physiological effects are mediated by the activation of AT1-subtype receptors. The receptors belong to the super-family of the G-protein-coupled receptors, and, in the case of AT1 receptors (AT1R), coupling occurs via Gq proteins. Consequently, stimulation of AT1 receptors activates phospholipase C, increases the levels of diacylglycerol (DAG) and inositol triphosphate (IP3), elevates the intracellular Ca\(^{2+}\) concentration, and activates several kinases, modulating cell functions. Angiotensin II acts as a mitogen in vascular smooth muscle cells by activating several signal transduction pathways, such as that of phospholipase C, phospholipase A\(_2\), and phospholipase D, as well as by activating a large number of kinases, such as tyrosine kinases, mitogen-activated protein kinases (MAPKs), c-src kinase, Janus-associated tyrosine kinase, and receptors with tyrosine-kinase activity. Angiotensin II also stimulates transcription factors, such as the activating protein, signal transduction and transcription activators (STATs), and the nuclear factor kappa B (NFkB).

Cloning of cDNA of the AT1 receptor provided the identification of a polymorphism in the nontranslated region 3' (A1166C), corresponding to an A to C transversion (adenine replaced by cytosine) in the position of the nucleotide 1166 of the mRNA sequence, resulting in 1 heterozygous (AC) and 2 homozygous (CC and AA) genotypes. The A allele that lacks the enzyme-restriction site is designated as the larger fragment whereas the C allele, which has an enzyme restriction site at nucleotide position 1166, is designated as the smaller fragment. The human AT1R gene, which mediates the major cardiovascular effects of angiotensin II, was cloned in 1992 and was present as a single-copy gene on human chromosome 3q 21–25 and spans about 60 kb including five exons and four introns. Exon sizes range from 59 to 2014 bp. Exon 5 is the largest and the only coding exon, while the first four exons encode a 59 untranslated region (UTR). AGTR1 is expressed in different organs including the heart, skeletal muscle, brain, human liver, lung, and adrenal gland. This receptor is included in the guanyl nucleotide binding protein (G-protein) coupled receptor super-family for which the intracellular messengers are phospholipase, calcium, and protein kinase. In humans, the AT, receptor is present predominately in vascular smooth muscle cells, and the AT2 receptor is present in the uterus, brain, and adrenal medulla. Both subtypes are also expressed in the adrenal cortex and kidney. The AT1 receptor, through which are exerted most of the actions of Ang II, is a G-protein-coupled receptor spanning seven transmembrane domains, and the cDNA and gene encoding human AT, have been cloned.

AT1R may be an important target for control of angiotensin II-dependent hypertension, as supported by the results of three studies. First, AT1R antagonists were shown to be effective antihypertensive agents. Second, a genome-wide scan suggested that the AT1R locus is the most significant contributor to hypertension in Finnish populations. Third, polymorphisms of the AT1R gene have been associated with hypertension. Although substitution of cytosine for adenine at position 1166 (A1166C) in the AT1R gene was associated with susceptibility to essential hypertension in French and Finnish populations, this finding was not confirmed in other ethnic groups, and thus these differences may be due to an ethnic variation. Paillard et al. (1999) found that AT1R sites on platelets are of limited density and that there is no effect of the genotype on receptor number or affinity. It also affects responses to losartan and angiotensin II. It has also been suggested that the A1166C polymorphism may be involved in the regulation of the expression of AT1R gene. Weak but significant linkage disequilibrium with a polymorphism in the promoter region of the AT1R gene and AT1R/A1166C has also been reported. Interethnic differences in cardiovascular diseases indicate the need to examine the association of AT1R gene polymorphism and hypertension in other populations.

The silent A1166C SNP in the AT1R gene has been associated with the severe form of essential hypertension, and in particular in drug-resistant hypertensive patients taking two or more antihypertensive drugs. The C allele was particularly over represented...
in Caucasian hypertensive subjects with a strong family history and it was also significantly more frequent in women with pregnancy-induced hypertension. A significant interaction between ACE I/D and AT1R+1166C polymorphisms in terms of influence on BP variation has been reported, although their linkage mechanism remains unclear. Henskens et al., (2003) confirmed an association of both these polymorphisms with BP in healthy normotensive subjects, although synergistic effects did not seem to be present. A higher prevalence of the AT1R CC genotype was found in Chinese hypertensive patients than in control subjects, whereas the +1166A/C genotype distribution did not differ between hypertensive and normotensive subjects from Japan. Tiet et al., 1998 showed a higher prevalence of C allele among female hypertensive patients than in control subjects but no such difference was observed in men. Szombathy et al., 1998 did not find any difference for this polymorphism in the AT1R gene between normotensive control subjects and subjects with resistant essential hypertension but high values of systolic BP were associated with the C allele in older overweight patients.

Lajemi et al., 2001 found that the 1166C allele in the AT1R gene influences the relationship between age and arterial pulse wave velocity in the additive effect with the -135A/G SNP in the ATIR gene. The C allele was also associated with aortic in both normotensive and hypertensive subjects but Girerd et al., 1998 did not find such a correlation with vascular hypertrophy in subjects with no evidence of cardiovascular disease. Takami et al., 1998 also reported an association between the C allele and left ventricular mass index, but in normotensive subjects without hypertrophic cardiomyopathy. These results are not in accordance with the studies of Hamon et al., 1997 and Ishanov et al., 1998 Hamon et al., 1997 also observed that the subjects homozygous for the AT1R CC genotype had a significantly lower ejection fraction than those with the A allele.

**Conclusion**

According to experiments and researches, genetic factors are involved in the process of hypertension including pathogenesis, diagnosis, treatment, and prevention of hypertension.

Considering the importance of genetic hypertension and the diversity of the related genes, evaluation of these genes and the study of new genes are necessary. It is hoped that by deducting related genes for essential hypertension, we be better able to diagnose those at risk and develop new treatments for these patients.

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