Hypertension is a common and complex human disease that causes significant morbidity and mortality worldwide. Unfortunately, despite recent advances in understanding and treating hypertension, its prevalence continues to rise. Across the globe, \( \approx 26\% \) of the adult population experiences hypertension, and Kearney et al\(^1\) estimate that this could rise to \( \approx 29\% \) by the year 2025. According to the American Heart Association, one third of the United States adult population is hypertensive, and one third of these remain undiagnosed. Hypertension is the most common risk factor for stroke and myocardial infarction and predisposes affected individuals to heart failure, ventricular arrhythmias, renal failure, blindness, and other serious medical problems, resulting in \( \approx 22\,000 \) deaths each year in the United States.

Essential hypertension accounts for \( \approx 90\% \) of hypertensive cases and is the 13th leading cause of death in the United States. Factors that may predispose a person to essential hypertension include weight, age, sex, ethnicity, physical activity, diet, cigarette smoke, stress, hormones, other medical conditions (eg, diabetes), and, of course, genetics. Indeed, studies of ambulatory blood pressure measurements in twins suggest that essential hypertension has a strong genetic component.\(^2\) However, the fact that patients often differentially respond to diverse classes of antihypertensive medications indicates that the etiology of hypertension likely varies considerably among patients, especially when large populations are considered.\(^3\) Consequently, researchers must attempt to tease out what must be a dynamic interplay among heterogeneous genetic backgrounds, diverse environmental factors, and differential etiologies in the pathogenesis of this disorder, all of which make attempts to understand the genetic basis of hypertension a challenge.

In this review, we will revisit the evidence supporting or refuting an association of the angiotensinogen (AGT) gene with hypertension. We will begin by describing the basics of the AGT gene and protein, followed by some of the known physiological roles of the classical and tissue renin–angiotensin system (RAS) in blood pressure regulation. A rationale for the implication of AGT in essential hypertension will be subsequently presented, emphasizing the role of linkage analyses and association studies. Particular attention will be given to the role of AGT gene polymorphisms in the etiology of essential hypertension. The reader is forewarned, however, that interpretation of the hypertension genetics literature is complicated, because it is replete with apparently conflicting results obtained largely from association studies using the case–control design. Whereas some of the disagreement among reports must arise from the factors discussed above (ie, genetic, etiologic, and environmental heterogeneity), a substantial proportion may be because of poorly defined inclusion criteria or inadequate appreciation of the importance of statistical power and replication. A critical review of the topic suggests that these last “two concepts [are] most commonly ignored in evaluating such studies.”\(^4\) We will conclude this review by delineating future research relating to AGT that would increase our understanding of its role in essential hypertension and that would perhaps guide development of effective therapeutic interventions.

The AGT Gene and Protein

Among candidate genes for essential hypertension, AGT was the first and remains perhaps “the most scrutinized” gene linked to the disease.\(^5\) It is expressed in multiple tissues, including liver, adipose tissue, heart, vessel wall, brain, and kidney; it is equally diverse in its cell specificity. Among the cell types expressing AGT are epithelial cells of the renal proximal tubule, hepatocytes of the liver, adipocytes in fat, and astrocytes and selected neurons in the brain. The human AGT gene, a member of the serpin gene superfamily, stretches over only \( \approx 12\,\text{kb} \) on chromosome 1 (1q42-q43) and contains 5 exons (starting at nucleotide 227 156 602 on chromosome 1 of the University of California Santa Cruz Human Genome Browser May 2004 Assembly). It is well conserved in vertebrates and also has homologs in invertebrates.\(^6\) Two important regulatory domains include the promoter found in the 1.2-kb region immediately upstream of the first exon and an enhancer found immediately downstream of the second polyadenylation site in the 3′ flanking region.\(^7\) Although this enhancer causes a 40-fold induction of expression using reporter constructs in human hepatocyte cell lines,
its importance in vivo has been disputed. The 485 amino acid (61 kDa) human AGT glycoprotein contains a signal peptide that is removed cotranslationally to yield the 452 amino acid substrate of renin. Whereas AGT is constitutively secreted, recent evidence also suggests that the protein may localize in the nucleus of some cells.

The Classical and Tissue RAS in Blood Pressure Regulation

The only known function of AGT is as substrate to renin. The N-terminal amino acids of mature AGT secreted by hepatocytes are cleaved intravascularly, first by renin released from juxtaglomerular cells, to yield the angiotensin-I decapeptide, and then by endothelial membrane–attached angiotensin-converting enzyme (ACE) to generate the angiotensin II (Ang II) octapeptide. Unlike the amino terminus of AGT, the remainder of the protein after cleavage by renin (termed des[AngI]-AGT) has no known physiological function. The renin–AGT enzymatic reaction is the rate-limiting step of the RAS cascade, and it is noteworthy that plasma AGT levels are close enough to the Michaelis constant for renin that small increases in either renin or AGT may increase Ang II production and alter blood pressure. This may be important, because many studies have associated variants in the AGT gene with circulating levels of AGT (discussed below). This is perhaps best exemplified by the elegant gene targeting studies of Kim et al., who demonstrated that altering the number of copies of the AGT gene affects plasma AGT and blood pressure. Increasing the copy number of AGT increased blood pressure (≈7 mm Hg per copy) while modestly increasing plasma AGT. The importance of elevated AGT is also illustrated by numerous transgenic mouse and rat models overexpressing the AGT and (renin) genes that exhibit markedly elevated blood pressure and hallmarks of hypertension (endothelial dysfunction, cardiac hypertrophy, and renal abnormalities).

Ang II is hypothesized to play a role in both the classical (synonyms in the literature include systemic, endocrine, or circulating) and tissue RAS (reviewed in Reference 14). It mediates most of its effects by binding Ang II type 1 receptors located in the central nervous system, heart, peripheral vasculature, kidney, and adrenal glands. Ang II stimulates, directly or indirectly (through aldosterone), renal sodium and water retention, a diuresic response (thirst and salt appetite), increased vascular tone, and vascular remodeling. ACE inhibitors and Ang II type 1 receptor blockers have beneficial effects (eg, preventing postinfarction cardiac remodeling) that extend beyond their ability to lower blood pressure. Similarly, these inhibitors can beneficially lower blood pressure in patients with a low index of circulating RAS activity. One explanation offered to account for these observations is the local production and action of angiotensin II in tissues (eg, heart, blood vessel wall, adrenal gland, kidney, and brain). Indeed, the beneficial effects of ACE inhibition seem to correlate better with the inhibition of ACE activity in tissues rather than in plasma. By definition, a tissue RAS exists in a tissue that expresses all of the mRNAs and proteins necessary to first generate Ang II de novo and then to exert the physiological effects of local Ang II. The two most intensely scrutinized tissue RAS are the brain and kidney, neither of which is thought to provide a major source of circulating AGT but rather produce abundant AGT locally. Studies in genetically manipulated mice suggest that nearly all, if not all, of circulating AGT is liver-derived. Nevertheless, some reports suggest that adipocyte-derived AGT may be relevant in the systemic circulation. This could be an important consideration in obesity and is in accordance with the strong correlations between obesity and plasma AGT and between obesity and hypertension.

AGT is abundantly expressed in glial cells throughout the brain and in selected neuronal populations located primarily within nuclei controlling cardiovascular function. Studies using reporter genes suggest that some of these neurons may also coexpress renin. Although the blood pressure and volume homeostatic actions of Ang II in the central nervous system are undisputed, few studies have examined the mechanisms of Ang II production within the brain. Studies using synthetic antisense oligonucleotides directed against AGT that were injected intracerebroventricularly into rats and other studies using transgenic rats expressing an antisense–AGT construct implicate the local production of Ang II in baseline blood pressure regulation and in hypertension. We showed that both glial and neuronal overexpression of renin and AGT leads to a modest elevation of blood pressure, and a glial-specific knockout of AGT in a model of systemic AGT overexpression with hypertension normalizes blood pressure. Ang II has been postulated to act as a neurotransmitter and has been detected in nuclei, as well as in axonal projections between nuclei controlling cardiovascular function reviewed in Reference 29). One implication of the colocalization of renin, AGT, and Ang II is the potential for intracellular synthesis of Ang II. Studies by us and others have revealed a novel form of renin mRNA expressed in the brain that presumably encodes intracellular active renin, and our recent studies provide the first evidence supporting a functional role for intracellular renin in the brain.

Like the brain, the kidney expresses all of the components of the RAS. That the concentration of Ang II in certain regions of the kidney, such as tubular fluid, is higher than can be accounted for by filtration of circulating Ang II implicates local synthesis. AGT is synthesized by proximal tubule cells and secreted into the tubular lumen, whereas renin is expressed in juxtaglomerular cells and secreted toward the renal interstitium. Intrarenal Ang II synthesis is stimulated, whereas renin is inhibited, by Ang II. This differential compartmentalization and regulation of renin and AGT makes it difficult to depict how Ang II is produced in the kidney. However, reports of renin expression in the proximal and connecting tubule, although controversial, along with abundant brush border ACE would provide the proximity of enzyme-substrate interactions necessary for generating Ang II in proximal tubule or tubular fluid. Indeed, targeted expression of both AGT and renin in the renal proximal tubule of transgenic mice leads to a modest elevation in arterial pressure. The importance of the intrarenal RAS in regulating arterial pressure, sodium reabsorption, and renal blood flow is well documented by physiological and pharmacological studies.
However, we were the first to convincingly demonstrate that elevated renal-specific expression of AGT causes systemic hypertension without a change in circulating Ang II,38 a finding that has now been replicated by another research team.39 This could have important implications for hypertension if renal-specific expression of AGT (or perhaps renin) is dysregulated. As discussed in detail below, some polymorphisms in AGT have been reported to affect transcriptional regulation of the gene, thus providing a potential mechanism for dysregulation to occur.40–42 It is, therefore, notable that patients with nonmodulating hypertension have been proposed to have dysregulated synthesis of intrarenal Ang II, and subjects carrying a specific haplotype of the AGT locus associated with increased transcriptional activity of AGT share phenotypic similarities with nonmodulators.43

The hypothesis that local production of Ang II in tissues may have physiological implications on blood pressure that occur independently of the systemic RAS seems to be gaining experimental support. Therefore the reader, when weighing the evidence supporting or refuting an association between AGT and hypertension, must consider the possibility that variants affecting the level of AGT expression may exert their effects in a tissue-specific manner and not necessarily by changing plasma AGT. Exactly which tissue-specific pathways regulate blood pressure under normotensive and hypertensive conditions in humans, the extent to which each tissue RCS is involved, the mechanisms by which their effects are mediated, and whether polymorphic variants in AGT are associated with tissue RCS or systemic RCS are questions that warrant further investigation. Like the multitude of factors influencing blood pressure in populations, the potential independence of tissue RCS may become a significant complication in studies linking AGT to hypertension.

A Genetic Link Between Hypertension and AGT?

Several early studies showing a positive correlation of plasma AGT levels with blood pressure,44 decreased blood pressure with administration of anti-AGT antibodies,45 increased blood pressure with injection of AGT,46 and elevated blood pressure in animals with AGT transgenes47 offered compelling evidence linking AGT to hypertension. However, the most compelling early genetic evidence implicating this gene in essential hypertension in humans came from the study of Jeunemaitre et al5 showing linkage between a marker downstream of the AGT gene and hypertension in affected sibships of Northern and Western European descent. Their use of an affected sibling strategy allowed them to accommodate the multifactorial etiology, delayed onset, and unclear mode of inheritance particular to common human disorders, such as essential hypertension. In brief, their strategy asked whether there was an excess sharing of specific AGT alleles among affected siblings as compared with levels expected based on chance (or random assortment) alone. Linkage was then independently verified in white European and African-Caribbean populations48,49 but was refuted in a separate large European cohort.50,51 Furthermore, linkage between AGT and pre-eclampsia (pregnancy-induced hypertension with proteinuria) was reported in populations in the United States, Japan, and Europe.52

The first screen for variants in the AGT gene by Jeunemaitre et al5 revealed a number of polymorphisms in the 5′ flanking region, exons, and introns of the gene. Variant alleles of two of these (T174M and M235T, which are generally in linkage disequilibrium) were found to be more prevalent in severely hypertensive cases than in controls, and the 235T variant, which also correlates with increased plasma AGT (≈20% higher in 235T homozygotes than in 235M homozygotes), was more frequently identified in pre-eclamptic patients.5,52 Soon after, many investigators focused their attention on the M235T variant, and, since then, the literature has become replete with contradictory reports arguing the importance of this allele. Both positive and negative associations were reported for white populations in North America, Australia, Japan, Europe, and Taiwan, and mainly negative associations were found in black populations in the Caribbean, United States, and Africa. A recent search of the PubMed database using the search terms “angiotensinogen” and “235” uncovered >210 published articles since 1993 (search terms were angiotensinogen in title AND 235 or M235 or T235 or M235T in text). This number increases to >575 when the search terms “angiotensinogen” and “polymorphism” are used (search terms were angiotensinogen AND polymorphism in text).

Some investigators shifted their focus from the structural and coding region of the gene to the 5′ flanking region when it was reported that the 235 variant was in nearly complete linkage disequilibrium with another variant at −6.53 Moreover, studies suggest that there is no difference in the kinetics of the renin–AGT reaction with either 235M or 235T AGT.54 Morgan et al40 and Inoue et al55 were the first to provide direct evidence for increased transcriptional activity of the −6A variant as compared with the −6G variant in the AGT promoter in a hepatocyte cell line, and, more importantly, higher expression of AGT mRNA in patients carrying the same allele. Large meta-analyses suggest that although some studies fail to associate AGT polymorphisms with hypertension, whites or Asians homozygous for the −6A/235T variant of AGT seem to have an increased relative risk for hypertension as compared with those who are homozygous for the −6G/235M AGT variant; the relative risk is not elevated for Africans.55 Whites and Asians homozygous for −6A/235T also seem to have elevated plasma AGT levels. It is noteworthy that 15% of whites, 53% of Japanese, and 67% of African-Caribbeans are homozygous for −6A/235T AGT.53

Several other single nucleotide polymorphisms (SNPs) in AGT have also been implicated in the pathogenesis of high blood pressure, including those at nucleotides −20, −217, −517, and −792. A polymorphism at the −20 position has been implicated to differentially modulate expression of AGT in HepG2 cells (Figure).41 Whereas the −20C allele has higher baseline promoter activity than the −20A allele, perhaps because of binding of the transcription factor USF (upstream stimulatory factor) to the −20C site, the −20A allele forms an estrogen response element that causes increased promoter activity in cells cotransfected with estrogen receptor-α.41 Analysis of this effect is made more complicated by the fact that the transcription factor Arp-1, an orphan member of the COUP-TF family, also binds to −20A and
antagonizes the effects of the estrogen receptor-α. Pregnant women homozygous for the −20C allele have lower plasma AGT than those homozygous for the −20A allele. Interestingly, whereas the −20C/−6A/235Thr haplotype would normally be considered the high plasma AGT haplotype, in pregnancy it is associated with a low plasma AGT state. The presence of the potential estrogen response element at position −20 was invoked to explain these results. Furthermore, the −20C allele (and the −20C/−6A haplotype) was associated with a blunted aldosterone response to Ang II and influenced the association between body mass index and ambulatory blood pressure. Like the −6 and −20 polymorphisms, a variant at position −217 affects baseline activity of the AGT promoter and is associated with hypertension. The frequency of the −217A variant is increased in black hypertensive patients, and the A at that position increases the binding of the C/EBP transcription factor. Finally, a variant at position −517 was also reported to strongly influence plasma AGT in a healthy French population.

More recently there has been a greater appreciation of the need to examine genetic variation in terms of haplotypes, the collection of SNPs present in a gene or region of the genome that are always, or nearly always, inherited together as a block. Nakajima et al identified 44 SNPs in the AGT gene and assembled a comprehensive haplotype map of AGT from whites and Japanese. Six major haplotypes of AGT account for most of the variation in the AGT gene, although the frequency of each differed substantially in the two populations. Zhu et al generated a haplotype map of each gene in the RAS and then performed association studies with individual SNPs and haplotype blocks in black and white hypertensive populations. Although a positive association was observed with several SNPs in AGT (as with other genes in the RAS), there was no transmission distortion of any particular haplotype for AGT. Contrasting results were obtained in another report evaluating the association between haplotype blocks of AGT and their interaction with the ACE locus in a Taiwanese population. These data revealed an increased transmission distortion of 5 (of 24) different AGT haplotypes in hypertensive subjects that carried a specific genotype at the ACE locus. Certain haplotypes of AGT were also associated with hypertension-related traits in the HyperGEN study.

Studies by Brand et al implicate a haplotype consisting of promoter, coding region, and 3’ flanking region variants that influence plasma AGT. Jeunemaitre et al identified 5 informative haplotypes of AGT after stratification for the −6A/235 allele associated previously with hypertension, two of which were associated with hypertension in whites but not Japanese. Nakajima et al reported 9 SNPs in the AGT promoter and defined 5 common haplotypes at 4 of these positions. An analysis of this region of the AGT promoter with transcription factor binding site prediction software suggests that there are putative binding sites that map to or near many of these SNPs (Figure). Therefore, the picture relating promoter variants to expression level may ultimately be much more complicated than perhaps originally anticipated. Moreover, because many of the functional studies reported to date have focused almost exclusively on cells derived from the liver, and only a few have examined AGT expression from other cellular sources, it is likely that the true complexity has yet to be revealed.

An Evolutionary Perspective
The “thrifty-genotype” hypothesis posits that certain genes evolved over vast expanses of time to benefit our ancestors in their ancestral environments but that recent changes in environment may render these previously advantageous polymorphisms now deleterious (reviewed in Reference 70 and with commentary related to hypertension in Reference 71). Because evolution is a continual process, however, there may be relatively recent mutations in such genes that attenuate these deleterious effects in individuals fortunate enough to have the newer alleles. In evolutionary history, higher levels of AGT (and, therefore, Ang II) may have been necessary in the salt-poor environment of sub-Saharan Africa. The −6A allele of AGT was identified as the ancestral allele based on its presence in nonhuman primates, and it is associated with increased transcriptional activity of AGT, as well as increased plasma AGT. A recent evolutionary study of AGT haplotypes in West Africans not only confirmed the ancestral allele, but it also suggested that over evolutionary history,

Schematic map of AGT. Structure of the human angiotensinogen gene with common SNPs depicted from the promoter region. Known transcription factor binding sites overlying SNPs are shown below the corresponding SNP. Putative transcription factor binding sites are in parentheses. UTR indicates untranslated region.
mutations accumulated in the AGT gene that increased circulating AGT levels. The environment in which Westerners now live where salt is replete has changed much faster than evolution, thereby making the ancestral alleles of AGT deleterious in our “burger and fries” environment.

**Perspectives**

As mentioned above, hundreds of association studies have been reported that have explored the importance of AGT polymorphisms for a variety of cardiovascular and noncardiovascular phenotypes. Other than hypertension, there has also been a large diversity in the cardiovascular phenotypes putatively associated with AGT variants. These include the response to antihypertensive drugs, stroke, obesity, diabetes, nephropathy, coronary artery disease, restenosis, and atrial fibrillation, each of which has a solid physiological rationale based on previous studies of the RAS. Noncardiovascular phenotypes examined for association with AGT include cognition and behavior, bipolar and panic disorder, asthma, substance abuse, life expectancy, polycystic ovary, athletic performance, and psoriasis. Some of these are based on physiological links that are not necessarily firmly established. When interpreting the >500 AGT association studies in the literature, one has to consider the strength of the association in terms of statistical power, replication in independent populations, and also the physiological rationale for the association.

In the end, one must ask whether there is a consensus on the importance of AGT as a genetic cause of hypertension. The easy answer is that a clear picture has yet to emerge that convincingly implicates AGT. However, one can safely take the view that variants in the AGT gene likely confer some small risk for hypertension, a finding supported by a number of meta-analyses. With the most frequently studied M235T variant of AGT, the T allele was associated with increased risk for hypertension in whites, but not blacks or Asians, in 1 meta-analysis, and with whites and Asians in the other. The T allele was also associated with a modest increase in circulating AGT. Despite some general agreement on the increased risk associated with the M235T variant of AGT, the T allele was associated with increased risk for hypertension in whites, but not blacks or Asians, in 1 meta-analysis, and with whites and Asians in the other. The T allele was also associated with a modest increase in circulating AGT. Despite some general agreement on the increased risk associated with the M235T variant of AGT, it is important to stress that when examined as a population, the data suggest only a very modest effect on total blood pressure. The GENIPER study of 2461 subjects recruited from 13 centers in Italy provides an example. First, it is not evolution, thereby making the ancestral alleles of AGT deleterious in our “burger and fries” environment.

Where does this leave us for the future? First, given the difficulty in unraveling the significance of genetic variation in hypertension susceptibility genes in humans, one must consider that remains a dearth of model systems that would allow investigators to study specific haplotypes using a controlled experimental approach. We previously examined the tissue- and cell-specific expression along with the physiological impact of the -6A/235T and -6G/235M variants of AGT in gene-targeted mouse models where direct comparisons between the allelic variants could be made. Admittedly, these studies were performed before there was an adequate appreciation for the overall haplotype structure of the gene. Therefore, the potential interactions between other polymorphisms, particularly in the promoter, could not be accounted for. Consequently, AGT haplotypes hypothesized to be associated with hypertension in different populations still need to be examined using defined experimental models where the interaction between genetic and environmental risk factors can be assessed. Jeunemaitre in his editorial comment on the GENIPER study suggested that “polymorphisms [may] exert their effect only in certain conditions, a ‘stress-the-genotype’ approach might help us unravel their true pathophysiological influence.” Whether experimental models using human cell lines or sophisticated gene targeted mouse models will aid in unraveling the complicated genetics of hypertension remains an unanswered question.

In the clinical arena, the rise of pharmacogenetics will become possible with the increasing ease and reliability of genetic tests. Classical pharmacogenomic studies focused largely on genes, such as members of the P450 cytochrome oxidase family affecting antihypertensive drug metabolism. More recently, attention has been focused on variants in individual candidate genes and their effects on antihypertensive therapy, which, in most cases, is small (reviewed in Reference 77). Whereas some positive results have been reported, further validation through replication is essential before any clinically relevant conclusions can be drawn. For example, the -6 SNP of AGT (essentially equivalent to M235T because of its linkage disequilibrium) has been reported to affect the systolic blood pressure response to a thiazide diuretic in black women and to atenolol in a Swedish population. It is conceivable that pharmacogenomic research may ultimately translate into an informed ability to custom tailor hypertension interventions, including the decision of whether to offer ACE inhibitors, Ang II type 1 receptor blockers, or other non-RAS therapies to a given individual or to guide lifestyle choices based on future risk of developing hypertension. Knowing a priori which therapies are most likely to provide effective blood pressure reduction for an individual with a specific genetic makeup would perhaps minimize the trial-and-error approach of current prescription strategies.
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Disclosures

None.

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