Angiotensinogen in Essential Hypertension: From Genetics to Nephrology

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Abstract. There is general consensus that genetic variation accounts in part for individual susceptibilities to essential hypertension. In marked contrast to classic mendelian disorders, in which genetic alterations produce a gain or loss of function, genetic determinants of essential hypertension, high blood pressure of unknown cause, are expected to be small, achieving significance through the cumulative effects of environmental exposure over the course of a lifetime. Whether and how genetic factors that contribute to common diseases can be identified remain unclear. Research on a link between angiotensinogen and essential hypertension illustrates a path that began in genetics and is now leading toward nephrology. Various challenges encountered along the way may prove to be characteristic features of genetic investigations of the pathogenesis of common diseases. The implication of a gene by statistical analysis is only the beginning of a protracted process of functional analysis at increasing levels of biologic integration. The ultimate goal is to develop an understanding of the manner in which genetic variation at a locus can affect a physiologic parameter and to extract from this inference new knowledge of significance for the prevention or treatment of disease.

Physical health depends on both genetic background and environmental exposure. Although it is agreed that genes contribute to the development of common diseases, the identification of such genes has proven both challenging and frustrating. For conditions as poorly defined as essential hypertension, an understanding of genetic predisposition may lead to better diagnoses, prevention, or treatments. Whatever strategy is used to identify such genetic determinants, the task of establishing causation and demonstrating the mechanism of disease remains daunting. Investigations of the link between angiotensinogen and essential hypertension illustrate this point. A similar course is being pursued by other investigators in at least two other instances in which linkage and/or association with essential hypertension has been indicated (1,2).

Development of a Genetic Hypothesis

Genetic Linkage

In 1989, we initiated genetic linkage studies of essential hypertension, in collaboration with Roger Williams, Pierre Corvol, and their colleagues. The three defining elements of the research program were a focus on components of the renin-angiotensin system (RAS), the use of microsatellite multiallelic polymorphisms as genetic markers, and the sampling and analysis of pairs of hypertensive siblings, to test for genetic linkage with a minimum of genetic assumptions. Variations in the number of copies of a simple DNA sequence motif at the locus of interest generate size differences that can be revealed by electrophoresis; these differences can be used to track shared inheritance of a chromosomal segment among siblings. Linkage is inferred when siblings inherit the same parental copy of the gene more often than expected on the basis of random segregation (Figure 1).

Studies of renin, using either biallelic markers (3) or a microsatellite (Jeanmaitre X, Soubrier F, Corvol P, Lalouel JM, unpublished observations), and angiotensin-converting enzyme (4) remained inconclusive. In contrast, significant genetic linkage was observed for angiotensinogen in two large series of independently ascertained sibling pairs, collected in Salt Lake City and Paris (5). In an attempt to define subsets with a greater likelihood of genetic determination, one of two criteria was used, i.e., the onset of hypertension before the age of 45 yr or the use of two antihypertensive medications (as a possible indicator of clinical severity). Greater statistical significance was achieved for each subset, despite the smaller sample sizes generated by these selection criteria. It was also noted that the greatest significance was achieved when the analysis was restricted to male siblings.

Angiotensinogen received less attention than other RAS components in previous genetic studies, despite warnings that it could well be an “underrated participant” in BP regulation.
and in the development of hypertension (6). It was not considered a candidate gene in early genetic studies of hypertensive rats. This relative lack of interest is attributable in part to the relative blandness of substrates, in contrast to enzymes. However, it also originates from the long-held view that angiotensinogen, which is present in plasma at concentrations long judged to be far in excess of the $K_m$ for its reaction with renin, could not contribute to BP regulation.

**Allelic Association**

Linkage between $AGT$ and essential hypertension suggested that genetic variations in the $AGT$ gene may affect individual predisposition to essential hypertension. The $AGT$ gene was scanned for the presence of such variation, and the frequencies of common genetic polymorphisms were compared between case and control subjects. Case subjects consisted of a proband randomly selected from each sibship with multiple hypertensive patients, whereas control subjects were selected from normotensive reference populations. The most significant observation was that, at residue 235 of angiotensinogen, an allele encoding the presence of threonine (T235) instead of methionine (M235) was more frequent among case subjects than among control subjects (Figure 2A). This association was significant for both the Salt Lake City and Paris samples. When data from nine European centers were pooled, however, no significant evidence of linkage could be obtained (9). The increased power expected for a larger sample may have been offset by increased heterogeneity of the patient populations sampled. Various attempts to replicate the association have been reported, with mixed results. Those studies were the subject of two meta-analyses, which confirmed significance in the aggregate and discussed features that affected the varying outcomes of the studies (10,11). These issues have been addressed by Corvol et al. (12).

**Association with Essential Hypertension in the Japanese Population**

An angiotensinogen-mediated predisposition to essential hypertension would be expected to affect sodium homeostasis and the regulation of fluid volume. Given the high prevalence of essential hypertension in Japan and the likely significance of dietary sodium for this population, a study was undertaken to test the association between T235 and essential hypertension among Japanese subjects. An initial report (13) revealed that T235 occurred at high frequency among Japanese subjects and was indeed associated with essential hypertension (Figure 2B).
With one exception (14), subsequent studies in Japan have confirmed this association (15–17).

Association with Preeclampsia

Plasma angiotensinogen levels increase severalfold in estrogenic states such as pregnancy or oral contraceptive use, and hypertension may be induced by such states. Indeed, preeclampsia is a common form of pregnancy-induced hypertension that has long been considered to involve a significant but unknown genetic component. T235 occurred at higher frequency among Caucasian women with preeclampsia than among control subjects, a finding that was corroborated in a small Japanese sample (18). This observation was confirmed in a larger sample of Caucasian patients (K. Ward, unpublished observations).

Haplotype Studies

If T235 serves only as a marker for one or more unknown functional variants in the gene, stronger association may be observed for subsets of T235 alleles defined through variations occurring at other sites in the gene. Common biallelic polymorphisms were identified by screening a 1200-nucleotide segment of the AGT promoter, all exons, the intron-exon junctions, and the 3'-untranslated segment of the gene. These segments were also sequenced for 16 individuals homozygous for T235 alleles. Multiple polymorphisms that occurred nonrandomly, and thus generated a limited number of haplotypes, were observed (19). The T235 alleles were subdivided into five subsets, whereas only one common variation subdivided the M235 allele (Figure 3). None of the subsets exhibited greater association with hypertension than that observed for T235 alone.

Another feature of this investigation of genetic polymorphism was the observation that several variants occurred in quasi-complete association. In particular, with few exceptions, T235 occurred with adenine (A) at position −6, whereas M235 occurred with guanine (G) at position −6. Therefore, all associations reported for T235 directly extend to A(−6).

Development of a Molecular Hypothesis

Testing of the Significance of the T235/M235 Polymorphism

The relevance of the RAS in arterial pressure (AP) regulation and the association of the T235 allele with higher plasma angiotensinogen levels suggested that, rather than serving as a marker for a neighboring gene, molecular variation in the angiotensinogen gene could mediate individual predisposition to essential hypertension. The complete association of the T235 and A(−6) alleles forced the consideration of several hypotheses. Either variant could be functionally implicated in predisposition or both could serve as markers for one or more unknown functional determinants.

The only well defined function of angiotensinogen is its role as a hormone precursor, serving as a substrate for renin in the formation of angiotensin I (AngI). On the basis of its sequence similarity, the protein belongs to the family of serine protease inhibitors, or serpins. The family includes inhibitors such as α1-antitrypsin and antithrombin III but also includes proteins that do not exhibit an inhibitor function, such as ovalbumin. Although there is no direct evidence that angiotensinogen can serve as a serine protease inhibitor, the matter has been the subject of repeated investigations (20).

The substitution of threonine for methionine at codon 235 does not, by itself, provide any functional clue. This residue occurs in a segment of the protein that demonstrates little conservation across species. Although the three-dimensional structure of angiotensinogen has not been established, some features can be inferred from consideration of the structures of other serpins, such as

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Haplotypes (Hap) of AGT in a case-control study of French subjects. No single haplotype appears to account for the T235 association (19).
α1-antitrypsin and antithrombin III (21). Codon 235 seems to occur in a part of the molecule that is not in close proximity to the amino terminus of the protein that encodes AngI. Structural models of renin complexed with peptide inhibitors demonstrate that the substrate assumes an extended conformation in the active cleft of the enzyme (22), which suggests that the amino-terminal portion of angiotensinogen protrudes in solution, with little secondary structure.

It remains conceivable that the T235/M235 polymorphism affects angiotensinogen function indirectly, by affecting protein conformation or glycosylation. To test this hypothesis, the protein, with either threonine or methionine at codon 235, was transiently expressed in cultured mammalian cells. Pulse-chase experiments demonstrated no difference between the two proteins with respect to glycosylation, secretion, or stability. Furthermore, the substitution had no detectable effect on the kinetics of enzymatic cleavage of the protein by purified recombinant human renin (23).

The T235/M235 polymorphism could also affect other biochemical characteristics and functions of angiotensinogen. A cysteine residue at position 232 may be involved in complex formation with other proteins, a process that may be differentially affected by the T235/M235 polymorphism (24). It has also been suggested that angiotensinogen may have a reactive center that mediates a serine protease inhibitor function (20); in vitro evidence was recently presented (25).

Testing of the Significance of Promoter Variants In Vitro

The G(−6)/A(−6) polymorphism occurs in the core promoter of the angiotensinogen gene. Mutations in such a gene region could affect DNA interactions with factors involved in transcription initiation and could thus affect the rate at which the gene is expressed. Markedly reduced expression of a reporter gene in HepG2 cells after removal of the residues from position −16 to position +44 indicated the critical significance of this segment of the AGT promoter (26). Other experiments have led to the suggestion that a ubiquitous transcription factor that binds to a segment of the mouse promoter spanning residues −6 to +22 could be an important determinant of transcriptional activity (27).

To test the significance of the G(−6)/A(−6) polymorphism in angiotensinogen expression, fusion genes were generated by placing a reporter gene (luciferase) under the control of segments of the human angiotensinogen promoter spanning either nucleotides −256 to +90 or nucleotides −70 to +90. Such fusion genes with either variant were transiently expressed in two types of mammalian cells, i.e., HepG2 and HEK293, which are transformed human hepatocyte and embryonic kidney cell lines, respectively. A large number of parallel experiments were performed in quadruplicate, using a variety of controls, to ensure the validity of the experimental comparisons. Further validation was provided by mutagenesis studies.

These experiments revealed consistent effects of the nucleotide substitution at position −6 on the transcriptional activity of the reporter (Figure 4). The differences observed were highly statistically significant, with significance levels ranging from $10^{-8}$ to $10^{-16}$. The magnitude of the observed effect, however, was modest; differences ranged from 20 to 50% when expressed on the observed scale of reporter activity, with $A(−6)$ exhibiting greater activity than $G(−6)$. Compared with effects typically analyzed in the transcription field, where losses or gains of certain DNA-protein interactions are examined, this seems to be a small effect. It is also small compared with the large differences noted between experiments, because the design affords little control of factors affecting transfection efficiency. If we follow the convention of expressing the data after standardization within experiments, the substitution effect can be expressed relative to its SD (as determined by $t$ test). The differences noted were equal to or exceeded 2 SD units (Figure 4). For statisticians, the magnitude of the difference between two means can be ascertained only by reference to the SD of the observations. When the data are considered in that context, the high level of significance observed reflects the high statistical power afforded by extensive experimental replication (23).

The differences in promoter activity associated with the nucleotide substitution suggested that the substitution could affect interactions with nuclear proteins involved in transcriptional control. DNA binding studies provided support for such specific interactions (23). The identification of specific factors that bind to this region of the gene could prove challenging, however, given the relatively low affinities revealed in these experiments.

This work illustrates some of the unique challenges that face investigators who attempt to elucidate the genetic determinants of common diseases. Graded differences that affect homeostatic regulation are not expected to produce the clean-cut qualitative differences that accompany mutations that produce gain or loss of function. It is also clear that causality cannot be inferred from such experiments. The novel observation that an additional common polymorphism at nucleotide 67 is in complete association with both the $G(−6)/A(−6)$ and M235/T235 polymorphisms (28) (Rohrwasser A, Lalouel JM, unpublished data) further supports the argument that these polymorphisms are associated with angiotensinogen expression in humans.

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Functional test of an AGT core promoter variant in vitro. Transactivation experiments revealed a significant effect of $G(−6)/A(−6)$ on a reporter gene transfected into cultured hepatocytes. Results are presented as means and SD, after standardization among multiple experiments. Luc, luciferase.
observations) further emphasizes this point. Because this polymorphism was also present in our expression studies (23), our published results reflect the compound effects of joint substitutions at nucleotides −6 and 67. In preliminary experiments, substitution at either site appears to affect transcription in vitro.

In Vivo Correlations

These promoter transactivation experiments support the conclusion that naturally occurring variations in the AGT promoter can affect basal transcription in vitro. As noted, “it is clearly not possible to directly extend the results of transfection experiments done with truncated AGT promoters in cultured cells to the function of an intact AGT gene at the level of the whole organism” (23). Two independent lines of evidence provide support for this hypothesis, however.

Smithies and colleagues (29,30) have provided direct evidence that a modest increase in AGT expression can lead to both increased plasma angiotensinogen levels and increased AP. Those authors used homologous recombination to inactivate or generate targeted gene duplication of the AGT gene in mice, generating animals with zero to four functional AGT genes. Of particular significance in the present context was the observation that animals with three functional AGT genes exhibited 10 to 20% elevations in plasma angiotensinogen levels and AP, compared with animals with two unaltered genes (Figure 5). The transgenic approach demonstrated a direct causal link between increased AGT expression and elevated BP. Furthermore, it demonstrated that a difference in expression of no more than 50% was sufficient to produce a detectable difference in AP.

The complete association between the polymorphism at nucleotide −6 and that at codon 235 suggested that the latter variation, which is present in transcribed mRNA, could serve as a marker to test for effects of the former on gene expression in tissue extracts. Although liver tissue was not readily available to us for this purpose, we had access to decidual tissue collected from women who had undergone elective abortions in the first trimester of pregnancy. We observed that, in human decidua, AGT expression was restricted to the smooth muscle cells of the spiral arteries (31). Total RNA isolated from decidual tissue from 39 women with the heterozygous M235/T235 genotype was tested for differential representation of AGT mRNA carrying either allele, using two different procedures, i.e., an allele-specific ligation assay and a single-nucleotide primer extension assay (31). Both assays revealed a significant excess of mRNA with T235 over mRNA with M235, providing an in vivo correlation in support of our hypothesis.

Molecular Hypothesis

The experimental evidence accumulated thus far affords the delineation of a working hypothesis. This hypothesis states that individual variations in the AGT gene that are associated with naturally occurring variations at nucleotides −6 and 67 and codon 235 impart modest differences in AGT gene expression. Individuals with the A(−6), C67, and T235 alleles may exhibit increased basal expression of the gene, which with time may lead to elevated baseline AP, compared with individuals without these alleles. The hypothesis that a chronic difference in AGT expression can lead to hypertension is supported by the animal experiments reported by Smithies and colleagues (29,30).

Significance of Angiotensinogen in Circulatory Homeostasis

Challenges in Proving Function and Cause

Genetic and molecular studies of the angiotensinogen gene support the hypothesis that genetic variations at this locus impart individual differences in AP regulation and the likelihood of developing essential hypertension. The finding that the linkage and association observed for such a phenotype are only modest could have been anticipated, given the high prevalence of essential hypertension, the ambiguous nature of its definition, the lack of specificity of the phenotype, its multifactorial causation, and the significance of environmental factors (such as diet and lifestyle) in its determination. For classic mendelian inheritance, support for linkage can be strengthened by tracing

Figure 5. Effect of AGT copy number on plasma angiotensinogen levels and arterial pressure in mice. The results reported by Smithies and colleagues (29,30) are compared with the association between the M235/T235 polymorphism and plasma angiotensinogen concentrations in human subjects.
single mutations in extended pedigrees. For common diseases, extending a collection of sibling pairs may not necessarily increase the statistical power, because enhanced ascertainment may be achieved only at the cost of increased heterogeneity in the sample.

Similarly, it is not altogether surprising that the actual molecular variants involved in the angiotensinogen gene, and the manner in which they affect circulatory homeostasis, cannot be established from direct observation of the mutation. Nonsense mutations, which signal gain or loss of function in classic mendelian disorders, were not found in the gene, nor were they expected for a genetic determinant of a common disease that must be both frequent in the population and of limited physiologic significance.

The molecular hypothesis outlined above suggests that genetic variations may affect angiotensinogen gene expression. It provides few clues, however, regarding the physiologic processes affected by the molecular variations observed in human subjects. To better understand the functional significance of individual variations for angiotensinogen expression, knowledge must advance at two levels, as follows. What physiologic functions are affected by changes in angiotensinogen expression? In what cell, in what organ, and under what circumstances can such differences in expression ultimately affect AP?

Evidence that Changes in Angiotensinogen Concentrations Can Affect the Rate of AngII Formation

The activity of the RAS depends on the rate of formation of AngII. Acute stimulation or depression of the system is mediated primarily by regulation of renin release and expression (32). In contrast to renin, the plasma concentrations of angiotensinogen vary slowly. However, in response to stimulation by estrogens, glucocorticoids, or thyroid hormone, plasma angiotensinogen levels can increase severalfold in 1 to 4 d. It was long thought that angiotensinogen was present in plasma at concentrations far in excess of the $K_m$ for its reaction with renin and that it was therefore not an important determinant of AngII formation. In more recent work performed with highly purified preparations of both enzyme and substrate, it was recognized that the plasma concentration of angiotensinogen was close to the $K_m$ for the reaction (33). It follows that changes in plasma angiotensinogen levels can indeed affect the rate of AngII formation, with other factors being equal. Another recent argument supporting the same conclusion is that, because of the high affinity but slow cleavage rate for the reaction between angiotensinogen and renin, the concentration of the former may affect the catabolism of the latter (34).

Evidence that Chronic Elevations in AngII Formation Can Lead to Increased AP

Angiotensinogen may contribute to AP regulation through extracellular fluid volume control. Gene titration experiments demonstrated that moderate but sustained increases in AGT expression led directly to increased AP (29,30), and kinetic considerations indicate that such increases could affect AngII formation.

Proof that chronic infusion of AngII at subpressor doses can lead to progressive increases in AP has been provided (35,36). Guyton long ago suggested that the increases in AP resulted from sodium retention, concluding that “the sodium-retaining effect of angiotensin almost certainly results from a direct effect of angiotensin on the kidney itself” (37, p 191).

Effects on Systemic and Local RAS

The systemic RAS and multiple local RAS are involved in AP control (32), and any of these systems can be affected by variation in angiotensinogen expression. The correlated increases in plasma angiotensinogen levels and AP observed in transgenic animals as the number of AGT genes is increased do not necessarily indicate a causal relationship between these two phenotypes. The detection of abundant angiotensinogen mRNA in proximal tubule and its stimulation by dietary salt (38,39) suggested a significant role for a local RAS in some aspects of proximal tubule function.

Significance of AngII in Renal Function

There is considerable evidence that AngII of both intrarenal and circulating origin affects renal function through effects on hemodynamics, the regulation of glomerular filtration, or the regulation of sodium reabsorption. Hall and Brands presented a very cogent review of the evidence supporting the conclusion that “the direct intrarenal actions of angiotensin II play a dominant role in long-term regulation of volume homeostasis and blood pressure” (40). It is often assumed that the sodium-retaining effects of AngII are mediated by stimulation of aldosterone secretion. Experimental models, however, convincingly demonstrated that, although minimal levels of aldosterone are necessary for normal physiologic function, “changes in aldosterone secretion are normally not essential in maintaining sodium balance or arterial pressure during fluctuations in sodium intake” (40).

High Luminal Concentrations of AngII in Proximal Tubule

The finding by Seikaly et al. (41) that AngII is present in the luminal fluid of proximal tubule at concentrations that exceed those in plasma by at least 2 orders of magnitude suggested local formation of the peptide. Because the high molecular weight of angiotensinogen precludes its filtration through the glomerular membrane, tubular AngII is likely to be derived from angiotensinogen synthesized in the proximal tubule. Braam et al. (42) confirmed such high AngII concentrations. They also demonstrated that AngI was present in tubular fluid at concentrations similar to those of AngII. Local conversion is likely, because high levels of angiotensin-converting enzyme in proximal tubule have been reported (43). Whether angiotensinogen or angiotensin peptides are secreted by proximal tubule and whether filtered or locally produced renin catalyzes the reaction remains unsettled, as reviewed by Navar et al. (44). Formation of AngII at this site could be significant for the regulation of sodium reabsorption in this proximal segment of the nephron. Angiotensin receptors have been observed at both the basolateral and luminal sides of proximal tubule (45).
There is abundant evidence that luminal AngII stimulates the apical sodium/hydrogen-exchanger and possibly other transport systems in proximal tubule (46).

Whether AngII exerts any effects in more distal segments of the nephron is less clear, although several arguments suggest that it might (40,44). At least one report indicates that it may affect sodium reabsorption by an amiloride-sensitive channel in early collecting tubule (47). Angiotensin-converting enzyme is found in luminal fluid as well as at the luminal surface of distal sites (43), and AngII receptors have been mapped along most of the nephron, with evidence for their presence on the luminal side being provided by immunocytochemical analyses (48).

Elements of a Paracrine System along the Entire Nephron

Luminal Secretion of Angiotensinogen

Taken together, these observations point to the significance of angiotensinogen expression in proximal tubule for some aspects of tubular function, particularly in proximal tubule. While reexamining this issue, we obtained experimental evidence suggesting that a paracrine RAS may operate along the entire nephron (49).

Using mice as an experimental model, we confirmed that angiotensinogen was expressed in proximal tubule, in a dietary sodium-dependent manner (38), and in no other tubular segments. The granular appearance of angiotensinogen in the vicinity of periodic acid-Schiff-counterstained brush borders in proximal tubule sections suggested a secretory process (Figure 6A). When confluent monolayers of conditionally immortalized murine proximal tubule cells (50) were grown on permeable membranes, angiotensinogen was reproducibly detected only in the apical chamber (Figure 6B). These in vitro data suggested secretion into the tubular lumen in vivo; some evidence for this process was previously provided by micropuncture data presented in abstract form (51). Because angiotensinogen is not filtered, indirect evidence for luminal secretion can be sought by testing for the presence of angiotensinogen in the final urine. Angiotensinogen was detected in the urine of mice and human subjects by an indirect immunoassay that measured uncleaved protein. No significant amounts of material could be detected for mice fed a high-sodium diet, whereas amounts reaching nanomolar concentrations were observed after sodium restriction. Taken together, these observations support the conclusion that angiotensinogen is secreted in the tubular fluid of proximal tubule, as a function of dietary sodium, and transits through the entire nephron. This contrasts with the fact that neither renin protein nor renin mRNA could be observed in proximal tubule under conditions of either high or low sodium intake, whether tissues or murine proximal tubule cells were examined.

Renin Expression in Connecting Tubule

The presence of angiotensinogen in tubular fluid throughout the nephron could represent elimination or delivery to a distal site. Immunoreactive renin has occasionally been noted in tubular segments (52–55), but those observations have been interpreted either as artifactual or as evidence for nonspecific reuptake of filtered renin. We examined renin distribution in the kidneys of animals after acute changes in dietary sodium levels. In sodium-restricted animals, specific immunostaining was observed in cortical clusters of open tubular segments that, on the basis of their distribution and morphologic features, were identified as the arcades formed by merging connecting tubules of midcortical and deep nephrons (Figure 7A). Staining was predominantly observed at the apical side of some of the cells in these tubular sections. Staining for renin and H\textsuperscript{+}-ATPase in consecutive serial sections revealed that the two stains were mutually exclusive, supporting the conclusion that renin expression was restricted to principal cells of the connecting tubule (Figure 7B).

Local synthesis was demonstrated using two approaches, i.e., microdissection of tubular segments followed by reverse-transcription-PCR and in situ reverse transcription-PCR (49). For each approach, four independent experiments were performed and yielded concordant results. Detection of renin mRNA required induction by either acute sodium restriction or...
amiloride inhibition of sodium reabsorption in animals after the switch to a high-sodium diet. To obtain additional evidence for the local production of renin, segments of midcortical tubular arcades were microdissected from sodium-restricted animals and the cells were dissociated, washed, transferred to charged membranes, and probed for the presence of immunoreactive renin (56). Connecting tubule cells displayed immunoreactive halos similar to those observed for positive control cells (Chinese hamster ovary cells stably transformed for mouse renin), in contrast to negative control cells (Chinese hamster ovary cells stably transformed for human angiotensinogen) (49).

**Significance of a Tubular RAS along the Entire Nephron**

These observations provide direct evidence for a RAS extending from proximal to distal segments of the nephron (Figure 8). The regulation and function of this paracrine system are only partly understood at this time. The contrasted distribution of angiotensinogen in proximal tubule and renin in connecting tubules suggests both regional and coordinated functions.

In proximal tubule, the partition of circulating and luminal angiotensinogen across the glomerular membrane contrasts with the filtration of systemic renin. This topographic organization affords both independent regulation of the rate of angiotensin formation through control of substrate availability in each compartment and coordinated regulation through delivery of active enzyme. Assuming that the dominant role of the circulating RAS is in regulating vascular tone, whereas that of AngII formation in proximal tubule is via its effect on bulk sodium reabsorption, this disposition may be critical for circulatory homeostasis, by affording coordinated regulation of both vascular tone and plasma volume.

The macula densa provides a mechanism to relate renin release by the juxtaglomerular apparatus into the circulation to sodium delivery downstream from the proximal tubule. Aspects of this feedback regulation have been well characterized (57). Although it is self-evident that sodium excretion depends on fine reabsorption in terminal segments of the nephron, it is unclear whether and how the RAS is involved. There is ample indirect evidence that intrarenal generation of AngII plays a critical role in circulatory homeostasis in response to changes in dietary sodium (40). The synthesis and secretion of renin in connecting tubules and the action of renin on luminal angiotensinogen originating in proximal tubule would allow AngII to participate in the regulation of sodium excretion through its effect on sodium reabsorption in the collecting duct. Interactions between substrate originating in proximal tubule and renin generated in connecting tubule may also provide a mechanism to coordinate bulk and fine sodium reabsorption at these

![Figure 7. Renin in connecting tubule. (A) Immunoreactive renin is observed in tubular arcades of midcortical nephrons. (B and C) Renin immunostaining is observed in principal cells of connecting tubule (B) but not in intercalated cells staining for H⁺-ATPase (C).](image)

![Figure 8. Elements of a paracrine tubular renin-angiotensin system. Filtered renin (R) of systemic origin may interact with angiotensinogen (A) secreted by proximal tubule (PT) to regulate bulk sodium reabsorption. Connecting tubule (CNT) renin may interact with angiotensinogen of proximal origin to regulate fine sodium reabsorption.](image)
two sites, in a manner that remains to be characterized. Al-
though speculative, these considerations can serve as a basis
for future experimental studies.

Conclusion
The identification of genetic determinants of essential hy-
pertension might prove more challenging than expected from
the remarkable advances that have been made regarding the
biologic basis of inherited mendelian disorders. The reason
evidently is that essential hypertension does not follow simple
mendelian inheritance. Modest genetic effects are evidently
relevant only with integration of various environmental exposures
with time. Such subtle individual differences may represent the
key to disease mechanisms and specific therapeutic inter-
ventions, however. When the bases of individual differences in
physiologic regulation are understood, risk factors may be
better correlated with biologic parameters that more directly
reflect genetic effects, and drugs may be developed to target
such individual differences. The key to effective diagnosis and
treatment remains the identification of inborn differences that
account for innate individual tendencies to deviate from good
health.

References
1. Ferrandi M, Bianchi G: Genetic mechanisms underlying the
regulation of urinary sodium excretion and arterial blood pres-
2000
2. Siffert W: G protein beta3 subunit 825T allele, hypertension,
obesity, and diabetic nephropathy. Nephrol Dial Transplant 15:
1298–1306, 2000
3. Jeunemaitre X, Rigat B, Charru A, Houot AM, Soubrier F,
Corvol P: Sib pair linkage analysis of renin gene haplotypes in
Absence of linkage between the angiotensin converting enzyme
locus and human essential hypertension. Nat Genet 1: 72–75,
1992
CS, Charru A, Hunt SC, Hopkins PN, Williams RR, Lalouel JM,
et al.: Molecular basis of human hypertension: Role of angio-
6. Menard J, el Amrani AL, Savoie F, Bouhnik J: Angiotensinogen:
An attractive and underrated participant in hypertension and
7. Caulfield M, Lavender P, Farrall M, Munroe P, Lawson M,
Turner P, Clark AJ: Linkage of the angiotensinogen gene to
8. Caulfield M, Lavender P, Newell-Price J, Kamdar S, Farrall M,
Clark AJ: Angiotensinogen in human essential hypertension.
Hypertension 28: 1123–1125, 1996
9. Brand E, Chatelain N, Keavney B, Caulfield M, Citterio L,
Connell J, Grobbée D, Schmidt S, Schunkert H, Schuster H,
Sharma AM, Soubrier F: Evaluation of the angiotensinogen locus
in human essential hypertension: A European study. Hyperten-
sion 31: 725–729, 1998
between the angiotensinogen 235T-variant and essential hyper-
tension in whites: A systematic review and methodological ap-
R, Fagard R: M235T angiotensinogen gene polymorphism and
12. Corvol P, Persu A, Gimenez-Roqueplo AP, Jeunemaitre X:
Seven lessons from two candidate genes in human essential hy-
pertension: Angiotensinogen and epithelial sodium channel.
Hypertension 33: 1324–1331, 1999
K, Lalouel JM: Angiotensinogen as a risk factor for essential
y: Angiotensinogen gene and essential hypertension in the Japa-
inese: Extensive association study and meta-analysis on six
reported studies. J Hypertens 17: 757–763, 1999
15. Iwai N, Ohmichi N, Nakamura Y, Mitsumami K, Kinoshita M:
Molecular variants of the angiotensinogen gene and hypertension
Ogihara T: Association analysis of a polymorphism of the an-
Hypertens 8: 521–524, 1994
T, Matsuo M: Effect of the angiotensinogen gene Met235→Thr
variant on blood pressure and other cardiovascular risk factors in
A molecular variant of angiotensinogen associated with pre-
M, Sharma AM, Gimenez-Roqueplo AP, Hata A, Corvol P,
Lalouel JM: Haplotype of angiotensinogen in essential hyper-
21. Carrell RW, Stein PE, Fermi G, Wardell MR: Biological impli-
cations of a 3 Å structure of dimeric antithrombin. Structure 2:
257–270, 1994
22. Dhanaraj V, Dealwis CG, Frazao C, Sibanda BL, Tickle IJ, Cooper
peptide-inhibitor complexes define the structural basis of specifici-
ity for human and mouse renins. Nature (Lond) 357: 466–472,
1992
23. Inoue I, Nakajima T, Williams CS, Quackenbush J, Puryear R,
Powers M, Cheng T, Ludwig EH, Sharma AM, Hata A, Jeune-
maitre X, Lalouel JM: A nucleotide substitution in the promoter of
human angiotensinogen is associated with essential hypertension
and affects basal transcription in vitro. J Clin Invest 99:
1786–1797, 1997
Jeunemaitre X: Role of cysteine residues in human angiotensino-
gen- Cys232 is required for angiotensinogen-pro major basic
protein complex formation. J Biol Chem 273: 34480–34487,
1998
D, Friedlein A, Langen H, Corvol P, Jeunemaitre X: Character-
ization of a human angiotensinogen cleaved in its reactive center
loop by a proteolytic activity from Chinese hamster ovary cells.
26. Fukamizu A, Takahashi S, Seo MS, Tada M, Tamimoto K,
Uehara S, Murakami K: Structure and expression of the human
angiotensinogen gene: Identification of a unique and highly
