Seven Lessons From Two Candidate Genes in Human Essential Hypertension

Angiotensinogen and Epithelial Sodium Channel

Pierre Corvol, Alexandre Persu, Anne-Paule Gimenez-Roqueplo, Xavier Jeunemaitre

Abstract—The candidate gene approach to understanding the genetics of human essential hypertension is discussed by analyzing the contribution of 2 genes, angiotensinogen (AGT) and epithelial amiloride-sensitive sodium channel (ENaC). From a large series of studies conducted in humans and animals, it appears that the AGT gene plays a significant but modest role in human blood pressure variance. Mutations of the β- and γ-ENaC subunits are responsible for Liddle’s syndrome, but the implication of the 3 ENaC subunits in essential hypertension is still questionable. Several lessons can be learned from these studies and applied to other candidate genes in essential hypertension: (1) Many linkage or association studies have a limited statistical power; (2) The genetic findings may vary greatly according to the populations studied; (3) There is a need for better phenotyping of the hypertensive population; (4) The causal relationship between molecular variants and hypertension is and will be difficult to establish firmly; (5) The contribution of genetic studies in rodents to the molecular genetics of human hypertension must be re-examined; (6) Most molecular variants lead to a low attributable risk in the population or a low individual effect at the individual level; and (7) It is too early to propose dietary recommendations and specific drug treatment according to patients’ genotypes. (Hypertension. 1999;33:1324-1331.)

Key Words: angiotensinogen ■ sodium channel ■ genetics ■ blood pressure ■ hypertension, essential

Considerable progress has been made during the past few years toward unraveling the molecular genetics of some rare, or extremely rare, monogenic forms of hypertension. The genes responsible for glucocorticoid-suppressible hyperaldosteronism,1 Liddle’s syndrome,2 and apparent mineralocorticoid excess3 have been identified. Even when the genes themselves have not yet been identified, the genetic loci have been mapped, as in Gordon’s syndrome,4 and in a pedigree affected with hypertension and brachydactyly.5 Considerable efforts have also been made to identify the genes responsible for the development of essential hypertension. The task is extremely difficult for several reasons. The heritability of hypertension is low (∼30% of blood pressure variance is attributable to genetic factors). “Hypertension” is an arbitrary definition and not a quantitative trait that appears relatively late in life. Nothing is known about the number of genes involved, their mode of transmission, their quantitative effect on blood pressure, their interaction with other genes, or their modulation by environmental factors. Parameters such as ethnicity and body weight increase the genetic heterogeneity and the difficulty of replication from one study to another.

The approach taken to date by the various groups working on the molecular genetics of human hypertension has been mainly to study candidate genes, i.e., genes that might contribute to abnormal blood pressure regulation because of their already established effect on cardiovascular or renal function. Many ligand/receptors, enzyme/substrates, and transporters contribute to the blood pressure, so that there are many candidate genes for hypertension. The number of studies designed to link or associate hypertension with gene variants has increased exponentially in recent years. But there is no clear picture as to why divergent results have been obtained. We have therefore evaluated the contribution of the candidate gene approach to our present understanding of the genetics of essential hypertension. The candidate gene strategy assumes that a given gene, or a set of genes involved in a specific function, might contribute to blood pressure variation. Linkage and/or association studies are conducted to test this a priori hypothesis.

Two candidate genes for human essential hypertension have been studied in depth, in particular by our group: the angiotensinogen (AGT) gene, which was the first gene shown to be linked to human hypertension,6 and the epithelial amiloride-sensitive sodium channel (ENaC). This gene is responsible for Liddle’s syndrome,2 which makes it a plausible candidate in essential hypertension. These 2 genes are critically involved, one in the activity of the renin system, and the other in renal sodium handling. The function of the variants discovered can be assessed in vitro systems. The present article reviews the contribution of these 2 genes to...
essential hypertension and the lessons that can be learned from them and applied to other candidate genes in hypertension.

**Angiotensinogen**

The well-documented effect on cardiovascular regulation makes the renin-angiotensin system a logical candidate for evaluation in hypertension. The cleavage of AGT by renin is the rate-limiting step in the cascade of enzymatic events leading to angiotensin. Plasma-AGT concentration is within the micromolar range, whereas the plasma-renin concentration is ~1000-fold lower. However, in addition to this large disparity between these 2 concentrations, AGT limits the amount of angiotensin I (Ang I) generated because its plasma concentration is not far from the \( K_m \) of renin. AGT is also synthesized and present in many tissues in addition to the liver, such as the brain, large arteries, kidneys, and adipocytes (see review in reference 7). Therefore, modest changes in plasma or tissue AGT could play a major role in the generation of Ang I, in fine tuning vascular resistance, and in controlling blood pressure.

**Essential Hypertension**

The first genetic study to show the potential role of the AGT gene in human essential hypertension was a sib-pair linkage analysis of 2 large populations of hypertensive sibships. A highly polymorphic GT microsatellite located in the 3′ region of the AGT gene was used to show genetic linkage between this gene and hypertension in severely hypertensive sib pairs (having a diastolic blood pressure >100 mm Hg, or taking 2 or more antihypertensive drugs). Mutations at the AGT locus were estimated to predispose at least 3% to 6% of the hypertensives younger than 60 years of age to hypertension.

The genomic sequences of the AGT locus were analyzed further and the coding and noncoding regions were examined for mutations. The initial 15 variants examined included 2 frequent alleles, T174 M and M235T, that were in complete linkage disequilibrium and associated with hypertension. The 235T polymorphism was more frequent in hypertensive probands, especially in the more severe index cases (0.50), than in controls (0.38). Lastly, plasma AGT was significantly elevated in patients bearing the 235T allele; a 10% increase in heterozygotes (MT) and a 20% increase in homozygotes (TT), versus MM homozygous individuals. Together, these results support the interpretation that molecular variants of AGT constitute inherited predispositions to essential hypertension in humans.

Several subsequent linkage studies showed a relationship between the AGT locus and high blood pressure. In the United Kingdom, Caulfield et al 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. The same group also found linkage between the AGT locus and hypertension in hypertensive sib pairs of African Caribbean origin. 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 multicentric 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.

Many association and case-control studies that involve blood pressure, hypertension, and AGT variants have been performed in addition to these linkage studies. Several variables must be taken into account in analyzing the published studies: (1) the nature of the AGT polymorphism studied (235T, 174 M, allele variants in the 5′ or 3′ region of the gene); (2) the type of study (general population, normotensive versus hypertensive subjects); (3) the clinical characteristics of the patients (age of onset and severity of hypertension, documentation of a family history of hypertension, drug treatment); and (4) ethnicity (Caucasians, Africans, Japanese, Chinese, etc). A recent review summarized the present findings, and 2 meta-analysis have been published. Meta-analyses, however, as stated by the authors themselves, should be interpreted with caution because they cannot evaluate the quality of the studies analyzed, and publication bias may lead to a lack of small studies with negative results. Keeping in mind this restriction, the 2 meta-analyses found a weak but significant association between the 235T-AGT allele and hypertension. Kunz et al found that the 235T allele was significantly associated with hypertension in 5493 white patients analyzed (odds ratio: 1.20; 95% confidence interval: 1.1–1.29; \( P < 0.0001 \)). The odds ratio increased modestly when patients with a positive family history or severe hypertension were considered. Staessen et al subsequently analyzed 32 case-control studies corresponding to 13760 patients and found an increased odds ratio conferred by the TT versus the MM genotype (odds ratio: 1.31; 95% confidence interval: 1.17–1.46, \( P < 0.001 \)). There was no association between the T allele and atherosclerotic or microvascular complications. Because the evidence incriminating AGT in essential hypertension remains statistical in nature, it is necessary to take other approaches to further document the relationship between AGT and blood pressure (Figure).

**Functionality of AGT Variants**

The next logical step is to look at the functions of the gene variants to see whether some of these can directly influence protein function and thereby the activity of the renin system. Two rare missense mutations of the AGT gene have been described recently. They provide a good model with which to evaluate their functional consequences in vitro and in vivo. A
mutation at the site where AGT is cleaved by renin (Leu<sup>10</sup> - Val<sub>21</sub>) was found in a patient with preeclampsia<sup>17</sup> and in 2 other unrelated severely hypertensive patients (X. Jeunemaitre, unpublished observations, 1999). Replacement of the leucine residue by a phenylalanine (L10P) resulted in a 2-fold increase in the catalytic activity of renin acting on this substrate. Angiotensin converting enzyme (ACE) had a 2-fold increase in catalytic activity on the corresponding angiotensin decapeptide Ang I (Phe<sub>10</sub>) than on natural Ang I. These kinetic differences probably increase the production of angiotensin II (Ang II) and stimulate the activity of the systemic and/or local renin systems. Gimenez-Roqueplo et al<sup>18</sup> identified a pedigree in which several members of the family were heterozygous for another AGT mutation in which a tyrosine residue is replaced by a cysteine at position 248 (Y248C). The heterozygous C248 individuals had 40% lower plasma AGT and Ang I production rate than the other members of the family. The expressed recombinant mutant was abnormally glycosylated and less was produced in culture.

The common 235T variant does not affect the K<sub>m</sub> of renin, and its secretion and metabolism are similar to that of 235 M AGT. However, the replacement of a methionine by a threonine residue is not neutral. Cohen et al<sup>19</sup> showed that 235 M and 235T AGT could be readily distinguished by a set of monoclonal antibodies, which allows plasma immunogenotyping 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. An extensive study of French and Japanese hypertensive patients found that a G→A substitution at position −6 upstream of the initial transcription site was present in almost complete linkage disequilibrium with the 235T allele.<sup>20</sup> The functionality of this A→6G substitution was evaluated in vitro by testing the corresponding promoter activity of the 2 variants.<sup>21</sup> This nucleotide substitution has a modest, but significant, effect on the basal rate of AGT transcription, which could account for the increase in plasma AGT in subjects with the 235T marker. An A→20C variant, also located in the promoter region, has an allele frequency of around 20% and may have an impact because it suppresses a consensus sequence for an estrogen receptor element.<sup>22</sup> Ishigami et al<sup>23</sup> analyzed 186 hypertensive Japanese patients and found a weak but significant correlation between A→20C, the plasma AGT concentration, and hypertension.

**AGT Gene Variants and Plasma and Tissue AGT**

**Plasma AGT**

The role of AGT in human hypertension was first suspected from an epidemiological study in which a strong correlation was found between plasma AGT concentration and blood pressure,<sup>24</sup> and from another study in which the offspring of hypertensive patients had elevated plasma AGT levels.<sup>25</sup> 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.<sup>6,26</sup> Bloem et al<sup>27</sup> 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<sup>28</sup> found a significant association with plasma AGT. Busjahn et al<sup>29</sup> reported a trend to a codominant 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 codominant and significant increase in plasma AGT concentration in patients bearing the M235T variant.<sup>30</sup>

**AGT mRNA**

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<sup>16</sup> described increased AGT 235T mRNA in the uterine spiral arteries of hypertensive women. The technique used specifically quantified the AGT mRNA transcribed from the 235T or 235 M alleles and showed that the expression of the 235T allele was ≈twice that of the 235 M allele. These results offered a plausible explanation for the association and linkage of the AGT locus with the occurrence of pregnancy-induced hypertension.<sup>32,33</sup>

**AGT and Renal Blood Flow Responses to Angiotensin II Infusion**

Williams and Hollenberg<sup>34</sup> described a phenotype called nonmodulation, in which the renal, vascular, and adrenal zona glomerulosa responsiveness to Ang II is altered under a high salt intake. This trait is genetically inherited, as up to 40% of essential hypertensive patients on a high salt diet show a reduced renal vascular response to Ang II.<sup>35</sup> The same group showed that the renal vascular response of homozygous patients carrying the 235T genotype to Ang II infusion was blunted in patients carrying the 235T genotype versus the heterozygous or homozygous genotype.<sup>36</sup> The interpretation was that patients carrying the 235T genotype produced more intrarenal Ang I, which could alter the normal renal response to Ang II in patients on a high salt intake. The same authors found recently a similar association in another set of hypertensive individuals, interacting with the ACE I/D polymorphism (G.H. Williams, personal communication, 1999).

**Epithelial Amiloride-Sensitive Na<sup>+</sup> Channel**

ENaC is the rate limiting step in Na<sup>+</sup> reabsorption by the renal distal tubule, and in other epithelia such as distal colon, salivary, and sweat glands (see review in reference 37). It consists of 3 homologous subunits α, β, and γ. Each subunit spans twice the plasma membrane and contains a large extracellular domain. The intracellular N and C domains are putative sites for the regulation of ENaC activity by phosphorylation. This channel has a remarkable specificity for sodium and lithium and is selectively blocked by amiloride and triamterene. Its activity is controlled by hormones such as aldosterone and vasopressin and by cytoplasmic regulatory
Several groups have identified molecular variants of mutation similar to that reported in Liddle’s syndrome, which affects the highly conserved PY motif at the carboxyl terminal end of the channel, which is altered or deleted in Liddle’s syndrome. We have screened 525 patients, including a subset of 101 subjects with low renin profile, but found no mutations located in the cytosolic carboxy-terminal part of the β-subunit of the ENaC (βENaC) that mutations located in the cytosolic carboxy-terminal part of the β-subunit of the ENaC (βENaC) are the cause of the disease in the original Liddle’s pedigree and in 3 other families.

Several mutations have been described in Liddle’s pedigrees, some in the β-subunit,2,39–42 others in the γ-subunit.43 All these mutations either abolish or modify a highly conserved PY motif present in their intracellular carboxyl terminus region, which in turn alters the binding of ENaC to its partner, Nedd4, a ubiquitin-like protein that contains a WW domain that can interact with the PY motif of ENaC.44,45 This leads to a low intracellular turn-over of the channel and to an increase in the number of active channels exposed at the apical membrane. The effect of these mutations have been studied in vitro by expressing the wild type or mutated ENaC subunits in Xenopus oocytes: all mutants described in Liddle’s syndrome produce a gain in function marked by an increase in the amiloride-sensitive sodium current. A direct in vivo confirmation of the constitutive activation of ENaC has been shown by Baker et al46 who found an increase in transmucosal nasal potential difference in patients affected with the disease versus nonaffected patients.

**Essential Hypertension**

Liddle’s syndrome could be considered to be the extreme of a pure salt-dependent form of hypertension. The salt-sensitivity of several forms of essential hypertension has been well documented, and several studies have shown that salt-sensitive hypertension is at least in part inherited.47 The hormonal features of Liddle’s syndrome, low plasma renin and low/normal aldosterone, have also been described in a subset of hypertensive patients, especially those of African origin. Because of the well documented role of ENaC in sodium reabsorption and its implication in essential hypertension, it was logical to consider ENaC as good candidate for essential hypertension and to screen hypertensive patients for mutations in the last exon of this subunit.50 However, Cui et al52 reported that protein kinase C did not inhibit ENaC activity in lymphocytes from patients bearing this variant, but the relevance of this observation to sodium homeostasis and blood pressure regulation is uncertain.

**βENaC**

Several groups have identified molecular variants of βENaC.42,48–51 All are missense mutations and none of them affects the highly conserved PY motif at the carboxyl terminal end of the channel, which is altered or deleted in Liddle’s syndrome. We have screened 525 patients, including a subset of 101 subjects with low renin profile, but found no mutation similar to that reported in Liddle’s syndrome, which makes a gross underestimation of the incidence of this disease unlikely.49 The same study revealed 7 missense mutations, almost all of them in patients of African descent. Subsequently, the most frequent variant in the C-terminus of the βENaC subunit (T594 M) was found to be 4 times more frequent in hypertensive black people resident in London than in their normotensive congener,50 but linkage of ENaC to hypertension was not found in another black population.51 The functionality of the T594 M variant is questionable because a small but not significant increase in Na+ current or 22Na uptake was observed after expression in Xenopus oocytes.49 However, Cui et al52 reported that protein kinase C did not inhibit ENaC activity in lymphocytes from patients bearing this variant, but the relevance of this observation to sodium homeostasis and blood pressure regulation is uncertain.

**γENaC**

Like βENaC, the γENaC subunit has been implicated because a missense mutation in the critical PY motif of the C-terminal end of the protein was found in a typical case of Liddle’s syndrome.43 We therefore screened the γENaC subunit for more subtle mutations in essential hypertension. The last exon of this subunit was screened in a large population of hypertensive and normotensive white patients and in hypertensive African Caribbean subjects.53 The search was extended to all exons of γENaC in a subset of 65 patients with a low-renin profile. Four neutral polymorphisms were found with similar frequencies in the hypertensive and normotensive whites and in the hypertensive subjects of African ancestry. Two rare mutations that affect the protein sequence were studied by expression of the gene variants in Xenopus oocytes, but they did not significantly increase the amiloride-sensitive Na+ current.53 This suggests that this subunit is not frequently involved in essential hypertension.

**αENaC**

We conducted a case-control study to evaluate the frequency of a new polymorphism (W493R) in a highly conserved region of the αENaC in 284 white patients.54 There was no statistical difference between the allele frequency of the W493R in hypertensive (irrespective of renin profile status) subjects and in controls. In addition, this variant did not increase the Na+ current after injection in Xenopus oocytes. Together, these results do not indicate that the W493R polymorphism of the αENaC gene is implicated in essential hypertension.

**Seven Lessons From the Analysis of AGT and ENaC Genes in Essential Hypertension**

Many Linkage or Association Studies Have Limited Statistical Power

A lack of power is one the major problem faced by all investigators in most genetic epidemiological studies. The affected sib-pair strategy is a robust method but has relatively low statistical power and requires large collections of well identified sibling pairs. Such families have been identified in only a few hypertension clinics or epidemiological surveys. Niu et al13 discussed the issue of statistical power in their...
linkage analysis of the AGT gene with hypertension in 310 hypertensive Chinese sibling pairs. They listed a series of parameters that should be taken into account to optimize statistical power. In particular, they emphasized the need to know the heritability of the trait (as) as well as the allele frequency of the markers in the population studied.

Association studies are easier to conduct and their methodology is well suited for detecting modest genetic effects within a complex and heterogeneous disease. The probability of detecting an association between a candidate gene and the disease depends on the allele frequency, the strength of its relationship with the disease, and the homogeneity of the population studied. A positive result might indicate a causal mutation or an allele in linkage disequilibrium with the causal mutation. A negative result has almost no power because it may be argued that other polymorphisms at the gene locus remain to be discovered. The main pitfall of this method is the possibility of false-positive results, mainly due to a population admixture and the failure to carefully select enough well-characterized cases and controls.

Interpretation of case-control studies is very often limited by the relatively small sample of patients and the fact that critical analysis of the statistical power of the study is not provided or discussed. Several studies have not the power to detect with enough statistical confidence a difference on genotype frequencies. There are even studies with power too provided or discussed. Several studies have not the power to detect with enough statistical confidence a difference on genotype frequencies. There are even studies with power too low for replicating the original findings on a candidate gene. For example, at least 400 cases and 400 controls are required to replicate our initial findings with 80% power to detect a difference in the 235T allele frequency from 0.38 to 0.46 between hypertensives and controls.

Genetic Findings May Vary Greatly According to the Populations Studied
The heritability of blood pressure and allele frequencies may vary considerably according to the population studied. For example, the AGT 235T allele is frequent in the Asian population (≈0.75) and largely predominant in the black population (0.90 to 0.95) versus the white population (≈0.40). As a consequence, false-positive results may arise from population stratification, but false-negative results may also be obtained in populations where this allele is largely predominant, due to the limited statistical power of the corresponding association studies. Similarly, allelic variations of the various subunits of ENaC are almost exclusively found in patients of African origin, which limits association studies to this ethnic group. This points out to the need for a rigorous homogeneity of patients with regard to ethnicity. Several genetic-epidemiological studies are performed in well-defined and limited geographic areas such as Iceland, Finland, and other genetic isolates to obtain maximal control of this parameter.

The allelic variation of AGT in humans may provide an interesting clue to its ancestral function. That 235T is the ancestral allele and that 235 M is the neomorph is suggested by extensive haplotype analysis of AGT in different populations, the great prevalence of the 235T allele in the majority of human populations, and the presence of the 235T allele in primates (monkeys). If this allele is associated with an increased activity of the renin-angiotensin-aldosterone system it could perhaps have conferred an advantage for retaining salt and for controlling body volume and blood pressure at a time when human access to salt was limited.

There Is a Need for Better Phenotyping of the Hypertensive Population
The vast majority of the published studies on AGT, ENaC, and other candidate genes have been performed on patients classified as having essential hypertension. Most of the patients studied are receiving treatment and their pretreatment blood pressure levels are not known. In addition, many variables that may affect blood pressure and should be taken into account such as age and gender of the patient, age at the time of discovery of hypertension, presence or absence of a documented familial history of hypertension, environmental factors (tobacco smoking, obesity, alcohol consumption, estrogen treatment) etc, are not reported or controlled. Better clinical and biochemical descriptions of hypertensive (and normotensive) populations would allow studies to be performed on more homogeneous populations and thereby increase their statistical power.

A detailed investigation of the physiological and biochemical parameters of well-characterized patients, in relationship with gene variants, might provide evidence for a functional link between genotypes and phenotypes. For example, the blunted renal vascular response to Ang II in homozygous nonmodulators carrying the 235T genotype versus the heterozygous or homozygous 235 M genotype suggests that the 235T allele is associated with increased intrarenal Ang II, thereby contributing to a disturbed renal physiology. In this study, obesity, which is itself genetically determined, interacts significantly with the AGT genotype and enhances the blunting of the renal vascular response. The link between obesity, AGT, and high blood pressure has yet to be proven, but several studies suggest such a relationship (see review in reference 14). A preliminary study by Baker et al shows increased urinary calcium excretion in patients bearing the T594 M variant. This suggests that compensatory mechanisms are activated to reduce sodium retention caused by the overactivity of this variant. Such studies could reveal unsuspected and interesting relationships between gene variants and intermediate phenotypes.

The Causal Relationship Between Molecular Variants and Hypertension Is and Will Be Difficult to Establish Firmly
The finding of a linkage or an association between a gene variant and hypertension is based on statistical analysis. The variant may be functional or a marker in strong linkage disequilibrium with a functional variant located on the same gene or even an adjacent gene. The finding of the functional variant is an important step because it establishes the functional link with high blood pressure or an intermediate phenotype and it allows further genetic studies with the mutated allele and not a distant genetic marker. Demonstrating the functionality of a molecular variant requires in vitro and in vivo studies. The functional allele of AGT may be the A→6G variant and not the 235T variant. The modest in vitro
increase in AGT transcription driven by the −6G variant compared with the −6A allele is in favor of this hypothesis, although it remains to be demonstrated by further experiments. It may well be that several variants of the promoter region of the gene interact together and modulate AGT gene expression.

The in vitro/in vivo demonstration of the functionality may prove to be difficult, even in cases where a simple cell assay is available. This is the case for ENaC, where it is possible to evaluate the functionality of the mutants detected by screening hypertensive patients for essential hypertension. Direct comparison can be made in vitro with the mutations observed in Liddle’s disease. None of the mutants found in essential hypertensive patients significantly increases sodium current. However, the expression of the ENaC channel subunits in Xenopus oocytes is a distant model from the activity of this channel in the mammalian nephron. If we postulate that a slight change in ENaC activity in humans results in only an excess renal reabsorption of 1 mmol/L per day, this would probably be missed by in vitro experiments. However, this extremely modest daily excess of sodium balance would provoke a cumulative gain of 365 mEq Na+ in a single year, which corresponds to >2 L of extracellular volume!

**The Contribution of Genetic Studies in Rodents to the Molecular Genetics of Human Hypertension Must Be Reexamined**

Candidate genes in human hypertension may also be implicated in selected rodent hypertensive strains. Conversely, it was hoped that a causal mutation found from genetic studies in spontaneously hypertensive rats (SHR) would also be involved in human hypertension. However, with the noticeable exceptions of the α-adducin gene and of the rat chromosome 10 locus, there has not yet been any definitive report of a mutated gene or loci responsible for high blood pressure in both humans and hypertensive animals.

Lodwick et al showed a cosegregation of the rat AGT locus with a modest increase in pulse pressure in F2 rats derived from a cross between SHR and normotensive Wistar-Kyoto rats (WKY). This locus accounted for ~20% of the genetic variance of this phenotype. The sequences of AGT mRNA in hypertensive and normotensive rats were identical but their AGT mRNA concentrations were different. No cosegregation was found between the AGT gene locus and blood pressure in F2 rats derived from a cross between stroke-prone SHR and WKY. Lastly, transfer of a segment of chromosome 19 that contains the AGT gene from the normotensive Brown Norway rat into SHR induced a blood pressure decrease, but the role of AGT itself was ruled out. These studies on SHR suggest that AGT is not a major genetic contributor to their hypertension.

Grunder et al and Kreutz et al investigated whether mutations in the 3 subunits of the rat ENaC contributed to high blood pressure in 5 rat models of genetic hypertension. No polymorphism cosegregating with hypertension was found, which excluded these proteins from being directly involved in hypertension.

The most informative experiments on the putative role of a gene in human hypertension have been performed in mice by the group of Smithies. They explored the possibility that a genetically determined elevated AGT concentration contributed to blood pressure with the use of an original strategy. Their hypothesis was developed from the original findings on the role of AGT in human hypertension. The human and mouse AGT sequences differ at position 235, so that it was not possible to introduce the human mutation into the mice AGT gene. Mice were genetically engineered to obtain different plasma concentrations of AGT, thereby mimicking the relative increase in plasma AGT resulting from the 235T allele effect. Targeted gene disruption and duplication were used to generate mice harboring 2, 3, or 4 copies of the AGT gene. Plasma AGT increased progressively but not linearly with the AGT gene copy number. The 3-copy mice have increases in plasma AGT similar to those of 235T allele patients, and the resulting 9 mm Hg increase in blood pressure is not far from the few mm Hg increase expected in humans. Renal blood flow varied inversely with the number of AGT copies as it is decreased in humans carrying the 235T allele. These results directly demonstrate that small increases in plasma AGT can quantitatively influence the fine control of renal vascular resistance and increase blood pressure in a gene dose-dependent manner. Similarly, ACE gene duplication in mice led to an increase in plasma ACE but no increase in blood pressure, which is in agreement with the lack of effect of the ACE gene in human studies. These elegant studies show that quantitative genetics, varying the gene copy number, can be used to assess rigorously the effects of genes in a complex quantitative trait (Figure).

**Most Molecular Variants Lead to a Low Attributable Risk in the Population or a Low Individual Effect at the Individual Level**

The risk of a patient affected with the mutation in monogenic forms of hypertension of developing high blood pressure is very high. An average increase of 20 mm Hg is associated with glucocorticoid suppressible hyperaldosteronism. The same applies to Liddle’s syndrome even though not every patient develops severe hypertension or the biological and hormonal features of the disease because of the interference of other genetic and environmental factors. The attributable risk in the general population, which depends on both the relative risk conferred by the mutation and its prevalence, is extremely low in monogenic forms of hypertension because of the very rare occurrence of the mutation.

The 2 meta-analyses of AGT polymorphism in hypertension showed that the relative risk of developing hypertension was 20% to 30% greater in subjects with the 235T allele than for those with the 235 M allele. This individual risk is low, but the attributable risk is not negligible because of the frequency of this allele. The genetic variance of blood pressure may result from small gene effects, each of them conferring a relatively low risk. The genotyping of thousands of single nucleotide polymorphisms will be soon feasible on DNA chips after multiplex polymerase chain reaction of a DNA sample. This technology will offer the possibility of studying many hypertension candidate genes at once and evaluating their cumulative effect on blood pressure. A combination of deleterious genes will probably account for
most of the genetics of essential hypertension in conjunction with environmental factors.

It Is too Early to Propose Dietary Recommendations and Specific Drug Treatment According to Patients’ Genotypes

AGT and ENaC are 2 genes that could be targeted for dietary control (salt restriction) and drug treatment (inhibitors of the renin-angiotensin system and amiloride) in patients carrying a susceptible allele. This is well-illustrated in the case of Liddle’s syndrome, in which the patients’ hypertension responds well to amiloride but to no other diuretic.

The M235T and the G—6A polymorphisms are associated with an increased plasma AGT, which could in turn result in a small increase in the rate of Ang II formation, especially in tissues where these proteins are rate limiting for Ang II generation. This genetically chronic overstimulation of the renin system then favors kidney sodium reabsorption, vascular hypertrophy or increased sympathetic nervous system activity, and predisposes the subject to hypertension and the development of common cardiovascular diseases. Understanding of these mechanisms may provide new tools for the diagnosis and treatment of individuals. A few positive reports have suggested a relationship between genetic polymorphism and responses to antihypertensive therapy.67 A study of 1509 white man and women patients participating in phase II of the Trials of Hypertension revealed a weak but significant decrease in diastolic blood pressure at 36 months, depending on the G—6A AGT polymorphism in the sodium restriction group.68 Defining the subset of individuals in which a gene or a combination of genes might play a more important role, such as salt sensitivity, is one of the important tasks for the near future.

References


Seven Lessons From Two Candidate Genes in Human Essential Hypertension: Angiotensinogen and Epithelial Sodium Channel
Pierre Corvol, Alexandre Persu, Anne-Paule Gimenez-Roqueplo and Xavier Jeunemaître

Hypertension. 1999;33:1324-1331
doi: 10.1161/01.HYP.33.6.1324

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/33/6/1324