Review

Genetic determinants of human hypertension

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ABSTRACT Hypertension is a common trait of multifactorial determination imparting an increased risk of myocardial infarction, stroke, and end-stage renal disease. The primary determinants of hypertension, as well as the factors which determine specific morbid sequelae, remain unknown in the vast majority of subjects. Knowledge that a large fraction of the interindividual variation in blood pressure is genetically determined suggests the use of genetic approaches in identification of primary determinants of human hypertension. The premise of such efforts is that identification of genetic determinants of human hypertension will provide key leads in unraveling the pathogenesis of this trait, will permit identification of individuals with specific underlying inherited predisposition, and ultimately may permit intervention in either preclinical or clinical stages with therapy tailored to these underlying abnormalities. All lines of evidence are concordant in supporting the influence of heredity on blood pressure: twin studies reveal a greater concordance of blood pressure in monozygotic twins than in dizygotic twins (1); epidemiologic studies reveal highly significant familial aggregation of blood pressure (2), which is not merely due to effects of shared environment since biological siblings have greater concordance of blood pressure than adoptive siblings raised in the same household (3). Such studies estimate that 20–40% of the variation in blood pressure in the population is genetically determined, with recent studies using more realistic models of inheritance providing generally higher estimates of heritability (4, 5).

Despite this evidence of a large effect of inheritance on blood pressure, these same studies suggest that blood pressure is commonly multifactorial in determination, since (i) blood pressure does not typically segregate in families in a fashion consistent with mendelian transmission and (ii) a variety of other factors such as salt intake, age, gender, and body mass can chronically influence blood pressure. It is popular to presume that the blood pressure in individual patients is due to the combined effects of variation at a number of blood pressure-determining loci, environmental factors, and demographic factors (Fig. 1). The number of loci in which variants affect blood pressure in humans, the magnitude of the effects imparted by each locus, and the model of inheritance at each trait locus are not readily estimated, providing little guidance as to how loci. These uncertainties pose strategic problems which are encountered in other complex traits such as diabetes, asthma, atherosclerosis, and neuropsychiatric disorders.

Approaches to Identification of Trait Loci

Owing to these uncertainties, it is difficult to determine the optimal approach to identification of trait loci affecting blood pressure in humans. A number of alternative approaches can be considered at present. While investigation of experimental animal models may provide important leads for human studies, it is readily apparent that there is no necessary relationship between genes identified in animal models and the pathogenesis of human disease, underscoring the necessity of human studies. Among approaches in humans, identification of subsets of the heterogeneous hypertensive population in which hypertension or an intermediate phenotype segregates as a mendelian trait may have the highest likelihood of short-term success, although these subsets may ultimately account for only a small fraction of affected subjects in the general population. Within the essential hypertensive population, approaches which may prove successful include linkage studies of affected relative pairs using either candidate genes or anonymous markers; association studies searching for linkage disequilibrium of alleles at candidate gene or anonymous marker loci and hypertension or intermediate phenotypes; and direct search for molecular variants in candidate genes followed by assessment of their functional significance by either expression of the gene product or further genetic studies. Further development of mapping methods which directly identify chromosomal segments of identity by descent (6) has the potential to streamline mapping approaches.

The various strengths and weaknesses of each of these approaches, as well as the types of populations that are most appropriate for each study design, can be debated. For example, association studies in typical urban populations in the United States commonly yield false-positive results owing to unrecognized underlying genetic differences between populations.

Abbreviations: ACE, angiotensin I-converting enzyme; GRA, glucocorticoid-remediable aldosteronism.
constituting cases and controls. Nonetheless, under true polygenic models of inheritance, in which inheritance at any trait locus has very small quantitative effects on the trait, such studies may provide the best opportunity to identify these loci. Methods which rely on distortion of the probability of transmission of variant alleles to affected offspring represent an approach to reduce the likelihood of such trouble-some false-positive studies (7). Similarly, the study of population isolates in which strong founder effects are likely to exist might provide a higher likelihood of success with disequilibrium studies. In this case the age of the isolate will directly influence the genetic distance over which disequilibrium extends. Newer isolates might be appropriate for initial mapping efforts (8), whereas older populations might provide mapping tools with high resolution (9).

For linkage studies, the appropriate phenotype for analysis (i.e., hypertension as a dichotomous trait versus quantitative blood pressure values versus intermediate phenotypes) and the population to be studied (i.e., population-based versus subjects ascertained for increased severity) are also issues for consideration. Since it is likely that the causation of hypertension in the population is heterogeneous, any success in increasing the degree of homogeneity can increase analytic power. This can in principle be achieved by use of intermediate phenotypes which identify specific subsets of the hypertensive population. The most rewarding of these are the mendelian forms of human hypertension, in which the effects of inheritance of single genes can be recognized and the power of now classical linkage approaches can be resurrected. In addition, a number of other intermediate phenotypes have been suggested; among these, increased erythrocyte sodium–lithium countertransport (10–12) and altered urinary kalikrein levels (13) have been proposed to show major gene determination. Other phenotypes, such as sensitivity of blood pressure to increases or decreases in sodium intake, have not demonstrated major gene effects (14).

In the setting of multifactorial determination, linkage studies using hypertension as a dichotomous trait in affected relative pairs retain considerable power to identify the chromosomal position of trait loci (15–18). The number of relative pairs required to detect such loci is dependent on numerous factors, including the true and unknown model of inheritance, the prevalence of blood pressure-altering variants in the population being studied, and the magnitude of the effect imparted by each trait locus. It is important to recognize that for this type of trait, the nature and prevalence of predisposing alleles may vary in different populations, so that results in, for example, Caucasians of northern European ancestry may not apply to other groups.

Candidate Genes from Physiologic Studies

A major concern in the genetic analysis of complex human traits is the relatively low power of linkage approaches to refine the chromosomal position of trait loci by linkage analysis, potentially leaving very large chromosomal regions harboring the trait locus; proceeding from such unrefined positional information to identification of functional mutations may prove extremely difficult in the absence of candidate genes in the linked region. It may be anticipated that determination of the nucleotide sequence of the human genome will greatly assist in identification of such candidates in the future.

For the present, the extensive investigation of the physiology of blood pressure regulation in humans and experimental animals provides a strong foundation for this effort, since these studies have identified a number of candidate gene loci for consideration. In the case of hypertension, such candidate genes are suggested not merely because they are expressed in, for example, the right tissue, but because investigation has demonstrated that altered function of the gene product can directly alter blood pressure. Because blood pressure is determined by the product of cardiac output and vascular resistance, key determinants of blood pressure may be presumed to act through these final common pathways. Physiologic pathways which are known to influence these parameters include the renin–angiotensin–aldosterone system (Fig. 2), which contributes to determination of both cardiac output, via effects on intravascular volume, and vascular resistance; the peripheral and central adrenergic pathways, which have dominant effects on cardiac inotropy, heart rate, and vascular resis-

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**Fig. 1.** Multifactorial model of blood pressure determination, demonstrating the potential influence of genes, environmental factors, and demographic factors on blood pressure. The potential interaction of these determining factors is represented by arrows linking different determinants.

**Fig. 2.** Schematic diagram of components of the renin–angiotensin system. Angiotensinogen is secreted by the liver. Active renin, secreted from the juxtaglomerular cells of the kidney, cleaves renin to angiotensin I (AI), which is cleaved by angiotensin I-converting enzyme (ACE) to produce the active hormone angiotensin II (AII). AII acts through specific receptors (AT1) to produce vasoconstriction and adrenal secretion of aldosterone. Aldosterone acts via the mineralocorticoid receptor in the distal nephron to increase sodium reabsorption, largely through its effect on the renal epithelial sodium channel. The locations in this pathway at which variants affecting blood pressure are located are indicated. GRA, glucocorticoid-remediable aldosteronism. [Reproduced with permission from ref. 19 (copyright 1995, Marcel Dekker, New York).]
tance; a variety of renal ion channels and transporters which determine net sodium reabsorption and hence intravascular volume; calcium channels/exchangers, whose activity influences vascular tone; and the nitric oxide pathway, which also affects vascular tone. A host of additional pathways which may tonically influence blood pressure have been reported. The importance of these systems in the regulation of blood pressure is demonstrated by the clinical efficacy of therapeutic agents which target various components of each of these systems. Early experience in this field has confirmed the relevance of candidate genes arising from physiologic studies to the genetics of human hypertension.

Molecular Genetic Studies of Human Hypertension

Linkage Studies in Essential Hypertension

Relatively few studies examining the potential role of candidate genes in the essential hypertensive population have been reported. No positive results solely relying on association studies have thus far proved replicable in independent patient populations, owing perhaps to the tendency of such approaches in urban populations to produce false-positive results. To date, linkage studies of hypertensive sibling pairs and a small number of candidate genes have been reported. Most of these studies have been performed in Caucasian hypertensive subjects ascertained either from the general population of Salt Lake City, Utah, or from a hypertension referral clinic in Paris, France. As might be expected from such different ascertainment schemes, the severity of the hypertension in the latter group is considerably greater than in the former. In general, the approach has been to identify informative genetic markers at candidate loci, either by identifying highly informative markers on contigs containing the candidate gene or by locating the gene on the human genetic map and employing closely linked flanking markers. With the increasing density of highly informative genetic markers on the human genetic map, it is virtually certain that highly informative surrogate markers for such candidate-gene linkage studies can be identified. These markers are then genotyped in family collections, and the experimentally observed concordance of alleles between affected sibs is compared with the allele sharing expected under independent segregation of trait and marker loci. When parental genotypes are available, one can establish that shared alleles are identical by descent (IBD) from a common parental allele; in this case, under the null hypothesis it is expected that sib pairs will share 0, 1, and 2 alleles IBD 25%, 50%, and 25% of the time, respectively. When parental data are missing and allele sharing is identical by state, in which case alleles either could be IBD or could represent duplicate copies of the same allele inherited from the parents, the expected allele sharing under the null hypothesis can be calculated from observed allele frequencies under the assumption of random mating. In either case, the results can be analyzed by a number of methods—for example, using a single-tailed test as a measure of significance.

To date genes encoding the sodium–hydrogen exchanger NHE-1 (20), renin (21), ACE (22), the angiotensin II receptor AT1 (23), and the SA gene (24) product have all been investigated in affected sibling pairs. Linkage studies of each of these genes have been negative, with no evidence of increased allele sharing among hypertensive sibling pairs (Table 1).

It is important to recognize the limitations of such negative studies. First, since these studies were all performed in Caucasian populations of northern European descent, little inference can be made regarding the potential role of variants in these genes in other populations. Second, it is important to acknowledge that variants in these genes could still impart significant effects on blood pressure but have not been detected in these studies. For example, these studies generally would have required a 5–10% excess in allele sharing IBD in hypertensive sib pairs in order to detect linkage at a significance level of 0.05. As a consequence, even variants with very large effect on blood pressure in the studied population could escape detection if they result in excess allele sharing below this threshold. Similarly, these studies will have low power to exclude effects of inheritance of common variants imparting small effects on blood pressure.

Angiotensinogen Variants in Human Hypertension

In contrast to these negative studies, recent studies with another candidate gene, encoding the renin substrate angiotensinogen, have provided compelling evidence for an effect of inherited variants in this gene on blood pressure in humans (25). The strength of this finding arises from the replication in independent patient samples of mutually reinforcing lines of evidence.

In both the Salt Lake City and Paris hypertensive sib pair sets, linkage of a highly informative marker at the angiotensinogen locus and hypertension was found in more severely affected sib pairs [defined as sibs having diastolic blood pressures > 100 mm Hg (1 mmHg = 133 Pa) or requiring two or more antihypertensive medications for control of blood pressure]. In both groups, there was >15% excess allele sharing in the hypertensive siblings compared with the allele sharing expected under independent assortment (P < 0.001). These findings motivated a search for molecular variants in the angiotensinogen gene; the prevalence of identified variants was then compared in cases and controls from these two populations. One of these identified variants, in which a threonine residue was substituted for methionine at codon 235 of the angiotensinogen gene, was found to be significantly more prevalent in hypertensive subjects than in normotensive subjects in both the Salt Lake City and Paris patient populations, with a prevalence of 36% in controls and 47% in unrelated index cases (P < 0.001) and 51% in more severely affected index cases (P < 0.001).

These findings supporting linkage and association of variants in the angiotensinogen gene with hypertension leave open the question of the mechanism by which such variants might affect blood pressure. The active hormone angiotensin II is produced from angiotensinogen by sequential cleavage steps by renin and ACE (Fig. 2). In response to contracted intravascular volume, the renal juxtaglomerular cells secrete more renin, resulting in increased formation of angiotensin II. Evidence suggesting a potential role of variation in plasma angiotensinogen levels in blood pressure determination has been present for many years (26, 27). In addition, however, it has long been recognized that plasma angiotensinogen levels are poised near the Km for cleavage by renin, with the consequence that alteration of angiotensinogen levels also affects the formation of angiotensin II. These findings raised the possibility that inherited vari-

Table 1. Linkage studies of candidate genes in hypertensive sibling pairs

<table>
<thead>
<tr>
<th>Locus</th>
<th>No. of hypertensive sib pairs</th>
<th>Alleles shared</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Expected</td>
<td>% excess</td>
<td>P value</td>
<td>Ref.</td>
<td></td>
</tr>
<tr>
<td>NHE-1</td>
<td>88</td>
<td>95</td>
<td>0</td>
<td>NS</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Renin</td>
<td>98</td>
<td>140</td>
<td>132</td>
<td>5.8</td>
<td>NS</td>
<td>21</td>
</tr>
<tr>
<td>ACE</td>
<td>237</td>
<td>255</td>
<td>255</td>
<td>0</td>
<td>NS</td>
<td>22</td>
</tr>
<tr>
<td>AT1</td>
<td>267</td>
<td>353</td>
<td>362</td>
<td>0</td>
<td>NS</td>
<td>23</td>
</tr>
<tr>
<td>SA</td>
<td>224</td>
<td>293</td>
<td>311</td>
<td>0</td>
<td>NS</td>
<td>24</td>
</tr>
<tr>
<td>Ang</td>
<td>379</td>
<td>487</td>
<td>466</td>
<td>5</td>
<td>0.02</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>110</td>
<td>159</td>
<td>135</td>
<td>17</td>
<td>&lt;0.001</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>NHE-1, sodium–hydrogen exchanger isoform 1; AT1, angiotensin II receptor type 1; Ang, angiotensinogen; NS, not significant.</td>
<td></td>
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ants at the angiotensinogen locus might act via alterations in plasma angiotensinogen levels. Examination revealed that the same variants in disequilibrium with hypertension were also associated with elevated plasma angiotensinogen levels; again, these findings were replicated in the Salt Lake City and Paris populations and were highly statistically significant (P < 0.0001) (25).

These findings constitute evidence that common variants at the angiotensinogen locus contribute to elevated blood pressure. At present, the identity of the true functional variant(s) at this locus is uncertain; T235 could represent such a functional variant or, alternatively, could be in disequilibrium with the true functional variants in these populations. As a consequence of this uncertainty, the proportion of alleles bearing T235 which impart an effect on blood pressure is unknown, and the magnitude of the effect imparted by each functional variant cannot be determined. Similarly, the mechanism by which such variants lead to altered angiotensinogen levels is unresolved. These variants could in principle act either by increasing the synthesis/secretion of angiotensinogen from the liver or by prolonging the plasma half-life of the protein. Further work will be required to distinguish between these possibilities and to establish the precise mechanisms by which variants affect blood pressure. At present, several important confirmations have appeared, ranging from linkage disequilibrium of angiotensinogen alleles and hypertension in several populations (28, 29) to demonstration of variation in blood pressure in mice as a function of the number of angiotensinogen gene copies (30). It appears that common variants at the angiotensinogen locus have a modest effect on blood pressure, as expected under a model of multifactorial determination.

Mendelian Forms of Human Hypertension. At present, four mendelian forms of human hypertension are recognized in which mutation in a single gene leads to elevated blood pressure in a high proportion of affected subjects: GRA (31), Liddle syndrome (pseudohypoaldosteronism) (32), pseudohypoaldosteronism type II (Gordon syndrome) (33), and the syndrome of apparent mineralocorticoid excess (AME) (31). Each of these disorders was initially recognized because of the presence of the precocious onset of hypertension in conjunction with abnormalities in electrolyte handling. As has been the case for mendelian forms of other complex traits such as colon cancer, breast cancer, and diabetes, genetic analysis of these single-gene forms of hypertension will likely be most rapid. Understanding these disorders will be of importance to the families that are affected and also will provide insight into basic mechanisms contributing to blood pressure variation which may prove broadly relevant to hypertension in the general population. The molecular bases of two of these disorders, GRA and Liddle syndrome, have been defined. The molecular bases of pseudohypoaldosteronism type II and AME are under investigation.

GRA. GRA is an autosomal dominant trait characterized by hypertension mediated via the mineralocorticoid receptor (34, 35). Affected subjects have elevated plasma aldosterone levels in conjunction with suppressed plasma renin activity and secrete high levels of two steroids that unaffacted individuals produce in negligible amounts: 18-oxocortisol and 18-hydroxycortisol (36–38). The physiologic sine qua non of GRA is now recognized to be the positive regulation of the secretion of aldosterone and these normal steroids by adrenocorticotropic hormone (ACTH) rather than the normal secretagogue for aldosterone, angiotensin II. A large GRA kindred phenotypically classified on the basis of 18-oxocortisol secretion demonstrated patent autosomal dominant segregation of this intermediate biochemical phenotype in the kindred (39). Linkage studies in this family demonstrated linkage of the gene causing GRA to a segment of chromosome 8 harboring a candidate gene, the aldosterone synthase locus (40). The aldosterone synthase gene is normally expressed in adrenal glomerulosa, whereas its gene product catalyzes the last two steps in the biosynthesis of aldosterone. This gene is 95% identical in DNA sequence to another gene involved in steroid biosynthesis, the steroid 11β-hydroxylase gene (41–43), which is involved in the biosynthesis of cortisol in adrenal fasciculata; both of these genes are present on chromosome 8, and it is now known that the two genes are very tightly linked, separated by only about 45 kb in genomic DNA (44). Investigation of these genes in this extended kindred demonstrated the presence of a novel mutation—unequal crossing-over between aldosterone synthase and 11-hydroxylase genes had resulted in a chimeric gene duplication fusing proximal sequences of 11-hydroxylase onto more distal sequences of aldosterone synthase (Fig. 3) (40). This mutation cosegregates with the disease at a recombination fraction of zero.

The physiologic consequences of this mutation explain the clinical features observed in affected individuals (40) (Fig. 4). Owing to S' regulatory sequences from the 11-hydroxylase gene, this chimeric gene is expected to be expressed in adrenal fasciculata under control of ACTH. Due to coding sequences from aldosterone synthase, however, it is anticipated that the encoded product will have aldosterone synthase enzymatic activity, with the consequence that this mutation results in ectopic expression of this critical enzyme in salt and water homeostasis. As a result, aldosterone is produced from corticosterone in adrenal fasciculata rather than adrenal glomerulosa; in addition, the signature steroids of GRA, 18-oxocortisol and 18-hydroxycortisol, are produced by the action of this enzyme on cortisol, which is normally synthesized in fasciculata. Aldosterone secretion leads to salt and water retention and, hence, plasma volume expansion. This volume expansion in turn suppresses plasma renin activity and turns off production of angiotensin II; however, this fails to suppress secretion of mineralocorticoids, since these are now under control of ACTH rather than angiotensin II; this unrestrained secretion of mineralocorticoids results in sustained volume expansion and hypertension.

Additional GRA kindreds have been examined, and all have been found to have similar chimeric gene duplications arising from unequal crossing-over (44, 45). The site of crossing-over has been variable, ranging over a 2-kb segment from the second intron to the fourth intron of these genes. In each case, exon 5 has been encoded by the aldosterone synthase gene, suggesting that this exon contains critical residues for producing aldosterone synthase enzymatic activity (44, 45). Biochemical studies have confirmed that introduction of

![Fig. 3. Chimeric gene duplications causing GRA. The intron–exon organization of normal aldosterone synthase and steroid 11β hydroxylase (11-OHase) genes are linked on chromosome 8 in the orientation shown. One of the chromosomal products of unequal crossing-over between these genes contains a gene duplication which fuses proximal sequences of 11-hydroxylase to distal sequences of aldosterone synthase. [Reproduced with permission from ref. 40 (copyright 1992, Macmillan Magazines, London).]](image-url)
exon 5 of aldosterone synthase into an otherwise normal 11-hydroxylase gene leads to aldosterone synthase enzymatic activity in the encoded product (45).

These findings establish the molecular basis of a mendelian form of hypertension. Mutations arising from unequal crossing-over are also found in thalassemias (46), red–green color vision blindness (47), and congenital adrenal hyperplasia due to 21-hydroxylase deficiency (48); however, these other disorders are typically characterized by loss of function due to gene deletion, rather than gain of function from the allele bearing duplications.

These findings afford the opportunity to define the consequences of inheritance of a specific mutation on blood pressure and clinical outcome. Early experience with this disease has revealed a striking predilection for one clinical outcome in these kindreds: cerebral hemorrhage at very early ages (39). This finding has been present in multiple individuals of a number of studied kindreds, suggesting that these mutations predispose to this particular clinical outcome.

Further investigation of kindreds with this disorder will also provide the opportunity to begin to define the quantitative effects of inheritance of this gene on blood pressure. Examination of the variability in phenotypic expression of affected subjects inheriting the same mutation by descent from a common ancestor may provide insight into the prevalence and action of other alleles in the population which affect blood pressure, and also afford new opportunities to study gene–environment interaction.

The molecular basis of GRA suggests that a simple test for these characteristic gene duplications can be applied to a small blood sample. This testing is likely to be applicable to pediatric subjects with unexplained hypertension, to subsets of the adult population with refractory hypertension or signs of aldosteronism in which aldosterone-producing adenoma and adrenal hyperplasia are excluded, and to at-risk relatives of affected subjects. The importance of making the diagnosis is that identified subjects are commonly classified as having refractory hypertension; however, once the diagnosis is made, they can be treated with a number of specific agents directed toward the primary abnormality. For example, pharmacologic modification of gene expression can be accomplished by administration of glucocorticoids: exogenous glucocorticoids suppress secretion of ACTH and thus turn off the expression of the chimeric gene. The efficacy of such treatment is demonstrated by the abrupt fall of sodium intake of aldosterone to subnormal levels, followed by the gradual return of normal glomerulosa function. Alternatively, these patients can be treated by blocking of action of mineralocorticoids at the receptor with competitive inhibitors such as spironolactone or by blocking the epithelial sodium channel with channel blockers such as amiloride.

**Liddle syndrome.** Liddle syndrome represents another autosomal dominant form of human hypertension (32). As in GRA, affected subjects have expanded plasma volume and suppressed plasma renin activity. In contrast, however, these subjects have suppressed aldosterone levels and no other circulating mineralocorticoids can be detected. The finding of hypokalemia in many affected subjects suggested that the hypertension in these patients might be mediated by excessive salt and water reabsorption in the distal nephron, where sodium reabsorption is indirectly coupled to potassium secretion.

Liddle’s original kindred was reexamined after the index case presented with end-stage renal disease (49). It was found that 18 subjects had the diagnosis of hypertension prior to age 20, suggesting use of this trait for linkage studies. Such studies ultimately identified linkage of Liddle syndrome to a short segment of chromosome 16, with a logarithm-of-odds score of 9.0 (50). In parallel, an amiloride-sensitive epithelial sodium channel was cloned: this channel was found to be composed of three subunits of similar structure—each contains two membrane-spanning domains, an extracellular loop, and intracellular amino and carboxyl termini (51, 52). The human homologs of these subunits were cloned, and one of these, the β subunit, was localized to the same segment of chromosome 16 as the gene for Liddle syndrome (50). Examination of this gene in five Liddle syndrome kindreds demonstrated that all five had mutations in a 95-bp segment encoding the proximal segment of the cytoplasmic carboxyl terminus; these mutations were not found in 250 control subjects. All of these mutations introduce either premature stop codons or frameshift mutations which remove 45–75 amino acids of the normal carboxyl terminus of the encoded protein (50) (Fig. 5).

The finding of these characteristic mutations in the β subunit of the epithelial sodium channel in patients with Liddle syndrome, in conjunction with the physiologic characteristics of affected subjects, strongly suggested that these mutations result in constitutive activation of channel activity, leading to increased renal salt and water reabsorption independent of mineralocorticoid action, leading to volume expansion and hypertension. Expression of wild-type and mutant channels in Xenopus oocytes has permitted testing of this hypothesis. Expression of the α subunit of the epithelial sodium channel alone results in low levels of amiloride-sensitive sodium channel activity; this activity is greatly augmented by coexpression of the β and γ subunits, to levels which are similar to those seen in vivo (51, 52). Expression of heterotrimERIC channels containing the truncated β subunit results in a marked increase in sodium channel conductance, demonstrating that the molecular mechanism of Liddle syndrome is constitutive activation of this channel (53). Moreover, these findings implicate the cytoplasmic carboxyl terminus of the β subunit in the normal negative regulation of this channel (50, 53). Interestingly, similar truncation of the γ subunit, but not of the α subunit, also resulted in activation of channel activity (53). Coexpression of truncated β and γ subunits together resulted in further additive activation of the channel, indicating that each of these two subunits is involved in the normal negative regulation of channel activity and that these mechanisms must act independently or at least in parallel (53). The mechanism by which these carboxyl termini are involved in negative regulation of channel...
activity is unknown, but the ability to examine the functional consequences of these mutations provides the opportunity to biochemically dissect this system. The findings in patients with Liddle syndrome demonstrate the relevance of a newly identified gene family to blood pressure regulation in humans and suggest that other variants, common or rare, in these same genes could affect blood pressure. Patients with Liddle syndrome represent one form of salt-sensitive hypertension, and it is readily apparent how the effects of this gene can be influenced by an environment factor such as dietary salt intake. Since salt sensitivity in association with suppressed plasma renin activity and low aldosterone level is a common finding in human hypertension, particularly among African Americans (54), these observations in Liddle syndrome will motivate the detailed examination of genes encoding the subunits of the epithelial sodium channel, as well as of genes whose products regulate expression or activity of this channel, in hypertensive subjects.

The Future of Genetic Studies in Human Hypertension

As for other complex traits, the genetics of human hypertension is in its infancy. A small number of candidate genes and mendelian forms of the disease have been exploited. Interestingly, all three of the genes in which functional variants have been identified or inferred to date are part of the extended renin–angiotensin system and appear to impart effects on blood pressure through alteration of renal sodium reabsorption (Fig. 1). Similarly, virtually all of the other known sufficient causes of hypertension, such as renal artery stenosis and aldosterone-producing adenoma, act via this same mechanism. These findings support the notion of a common pathway to the development of hypertension in many settings, motivating further studies of the regulation of sodium homeostasis and intravascular volume. Nonetheless, few meaningful studies have been performed in other major pathways known to be involved in the tonic regulation of blood pressure, leaving open the possibility of major findings from either known or presently unknown physiologic systems.

There remains a pressing need for collection of relevant patient populations, particularly among minority groups. Identification of informative intermediate phenotypes, or additional mendelian forms of hypertension by family studies, would increase the chances of identification of trait loci. In the future, additional candidate-gene studies will be performed, and general linkage studies will also be carried out in a variety of populations. Genome-wide searches for linkage disequilibrium in population isolates represent another potential means of identifying new trait loci. Given the relatively low power of all such studies, assessment of the significance of identified loci will rely either on identification of underlying functional variants or on replication in independent patient sets. It remains clear that advances will be most rapid when genetic findings can be interpreted on the basis of established physiology, motivating the continued study of human and molecular physiology in this area.

In addition to these studies to identify determinants of hypertension, additional studies can be contemplated to identify determinants of particular clinical endpoints such as stroke, end stage renal disease, and myocardial infarction. The consolidation of health care into large networks in the United States may provide unique opportunities for ascertainment of large numbers of multiplex families with such specific end points. Genetic studies in such populations provide opportunities to determine whether specific genes affect clinical outcomes in individual patients; if found, these genes may provide opportunities for therapeutic intervention.

As functional variants are identified, their quantitative effects on blood pressure and their impact on clinical outcomes can be assessed by studies of the general population or by linkage in extended families. Opportunities will arise for evaluation of gene–gene and gene–environment interaction, and a renewed need for physiologic studies will be apparent. The opportunity to develop appropriate animal models based on identified functional variants, as well as to study the physiology of patients harboring different combinations of variants at trait loci, will likely provide important insights into the pathogenesis and treatment of hypertension and its complications.

Note Added in Proof. Since submission of this review, mutations in two additional genes have been implicated in human hypertension, demonstrating the rapid pace of this field. Mutations resulting in loss of function of the renal isozyme of 11β-hydroxysteroid dehydrogenase have been shown to cause the autosomal recessive syndrome of apparent mineralocorticoid excess (55). This enzyme normally converts cortisol to cortisone, thereby preventing corticosteroid activation of the type I mineralocorticoid receptor in the kidney. Loss of this enzymatic activity results in cortisol acting as a potent mineralocorticoid. A mutation truncating the cytoplasmic carboxyl terminus of the γ subunit of the epithelial sodium channel of the distal nephron has been shown to result in constitutive activation of channel activity, leading to increased sodium reabsorption and hypertension. This finding demonstrates genetic heterogeneity of Liddle syndrome (56).

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