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Molecular Genetics of Human Blood Pressure Variation

Richard P. Lifton

Hypertension is a common multifactorial vascular disorder of largely unknown cause. Recognition that hypertension is in part genetically determined has motivated studies to identify mutations that confer susceptibility. Thus far, mutations in at least 10 genes have been shown to alter blood pressure; most of these are rare mutations imparting large quantitative effects that either raise or lower blood pressure. These mutations alter blood pressure through a common pathway, changing salt and water reabsorption in the kidney. These findings demonstrate the utility of molecular genetic approaches to the understanding of blood pressure variation and may provide insight into the physiologic mechanisms underlying common forms of hypertension.

Elevated arterial blood pressure, or hypertension, affects 15 to 20% of the adult population in industrialized societies and is one of the principal independent risk factors for stroke, myocardial infarction, and end-stage renal disease. Because of this high prevalence, management of hypertension is thought to constitute the single largest health care expense in the United States.

Despite the public health importance of this trait, the pathogenesis of hypertension remains unknown in the overwhelming majority of patients. At face value, this ignorance may seem surprising, because the determinants of blood pressure are deceptively simple—blood pressure is the product of cardiac output and systemic vascular resistance to flow. However, although many of the physiologic systems involved in regulation of blood pressure have been identified, it has proved difficult to determine which systems have primary derangements in hypertensive patients, largely because of the complex interactions of these different physiologic pathways. As a result, we cannot reliably identify susceptible individuals in preclinical stages so that disease may be prevented, and our current treatment of hypertensive patients is necessarily empiric.

A variety of genetic, environmental, and demographic factors contribute to blood pressure variation, even in single individuals (*1*). The recognition that a substantial fraction of human blood pressure variation is genetically determined derives from studies comparing the blood pressures of monozygotic and dizygotic twins (*2*), from epidemiologic studies of familial aggregation of blood pressure (*3*), and from adoption studies comparing the blood pressure of biologic and adoptive siblings (*4*). These findings suggest the application of molecular genetic approaches to identify these underlying genetic components. The progress that has been made in this effort is reviewed here.

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Mendelian Forms of Hypertension

Recently, considerable effort has been devoted to the identification of genes responsible for mendelian, or single-gene, forms of severe hypertension. The reasons for this effort are several. First, the effects of segregation of single alleles in families can be discerned, thereby simplifying molecular genetic analysis. Second, because morbidity from hypertension is proportional to its severity, severe inherited forms may have particularly high attendant morbidity. Identification of the responsible genes may provide novel diagnostic tools as well as an opportunity for targeted therapeutic intervention. Third, the genes and physiologic pathways implicated in severe forms of hypertension may also be involved in the more modest forms of hypertension commonly seen in the general population. Finally, identification of these genes may provide insight into the pathogenesis of common forms of hypertension, which have been variously proposed to result from primary abnormalities in the brain, heart, vasculature, adrenal gland, liver, and kidney.

Glucocorticoid-remediable aldosteronism

(GRA). Aldosterone is a steroid hormone normally produced from the adrenal glomerulosa under the control of angiotensin II (AII). Through interaction with the mineralocorticoid receptor in renal tubular cells, aldosterone causes increased activity of the epithelial sodium channel, leading to net salt and water reabsorption (Fig. 1). This increased salt and water retention expands plasma volume and increases cardiac output by increasing venous blood return to the heart.

Glucocorticoid-remediable aldosteronism is an autosomal dominant trait characterized by moderate to severe hypertension that is detectable from birth onward (5). The hypertension in these patients is caused by constitutive secretion of aldosterone and perhaps additional adrenal mineralocorticoid hormones. The sine qua non of patients with GRA is that the secretion of aldosterone is regulated by adrenocorticotropic hormone (ACTH) rather than by its usual secretagogue AII. ACTH normally regulates secretion of the stress hormone cortisol from adrenal fasciculata.

Genetic analysis of GRA kindreds has revealed linkage of GRA to a segment of human chromosome 8 containing two genes whose products are known to be involved in adrenal steroid biosynthesis: aldosterone synthase and steroid 11β–hydroxylase (6). These two genes are highly similar in DNA sequence and have an identical intron-exon organization (7). In all GRA kindreds studied to date, disease chromosomes carry normal copies of the genes encoding aldosterone synthase and 11β–hydroxylase, but in addition they have a novel gene not found in unaffected people (Fig. 2A) (6, 8). These novel genes represent duplications arising from unequal crossing-over between the aldosterone synthase and 11β-hydroxylase genes, fusing 5′ regulatory sequences from the latter onto coding sequences of the former.

The structure of the chimeric gene explains the pathogenesis of the hypertension seen in GRA (6). By virtue of the 5′ regulatory element of the 11β-hydroxylase gene, aldosterone synthase gene expression and enzymatic activity are brought under the control of ACTH. This results in ectopic secretion of aldosterone from the adrenal fasciculata, which in turn leads to increased salt and water reabsorption, plasma volume expansion, and ultimately to a rise in blood pressure. This expanded plasma volume leads to suppression of renin secretion and reduced production of AII (Fig. 1); however, this does not turn off production of aldosterone, because secretion of this hormone is now controlled by ACTH.

Highly sensitive and specific genetic tests can now be used to screen for this characteristic chimeric gene (6, 8), and carriers can be treated by pharmacologic gene therapy (administration of physiologic doses of glucocorticoids suppress ACTH secretion, which in turn suppresses expression of the mutant gene) or by specific antagonists of the mineralocorticoid receptor or the epithelial sodium channel (ENaC).

Syndrome of apparent mineralocorticoid excess (AME). This syndrome is an autosomal recessive disorder characterized by early onset of moderate to severe hypertension (9). As in GRA, the hypertension in AME is caused by stimulation of the mineralocorticoid receptor (Fig. 1); however, in contrast to GRA patients, AME patients have very low levels of aldosterone. An exhaustive search for the mineralocorticoid responsible for the hypertension in these patients ultimately identified an unexpected culprit: the glucocorticoid hormone cortisol (10). This result was a surprise because this stress hormone normally has little mineralocorticoid activity in vivo.

The physiologic mechanism underlying this paradoxical activity has been elucidated. Aldosterone is normally a vastly more potent activator of the renal mineralocorticoid receptor than cortisol in vivo, but in vitro both steroids are potent activators of this receptor (11). The fact that cortisol normally circulates at concentrations that are orders of magnitude higher than those of aldosterone suggested the existence of a mechanism that normally prevents cortisol from activating the renal mineralocorticoid receptor in vivo. It is now known that cells that respond selectively to mineralocorticoids contain an enzyme, 11β-hydroxysteroid dehydrogenase, that metabolizes cortisol to cortisone, a steroid that is incapable of activating the mineralocorticoid receptor. This mechanism "protects" the mineralocorticoid receptor from cortisol, resulting in selective activation by aldosterone (12, 13).

The observations that cortisol has a markedly prolonged plasma half-life in patients with AME and that urine samples from AME patients show a striking elevation in the ratio of cortisol metabolites to cortisone metabolites led to the proposal that AME can arise from 11β-hydroxysteroid dehydrogenase deficiency (12, 13). This proposal was supported by the discovery that a compound derived from natural licorice, glycyrrhetinic acid, inhibits this

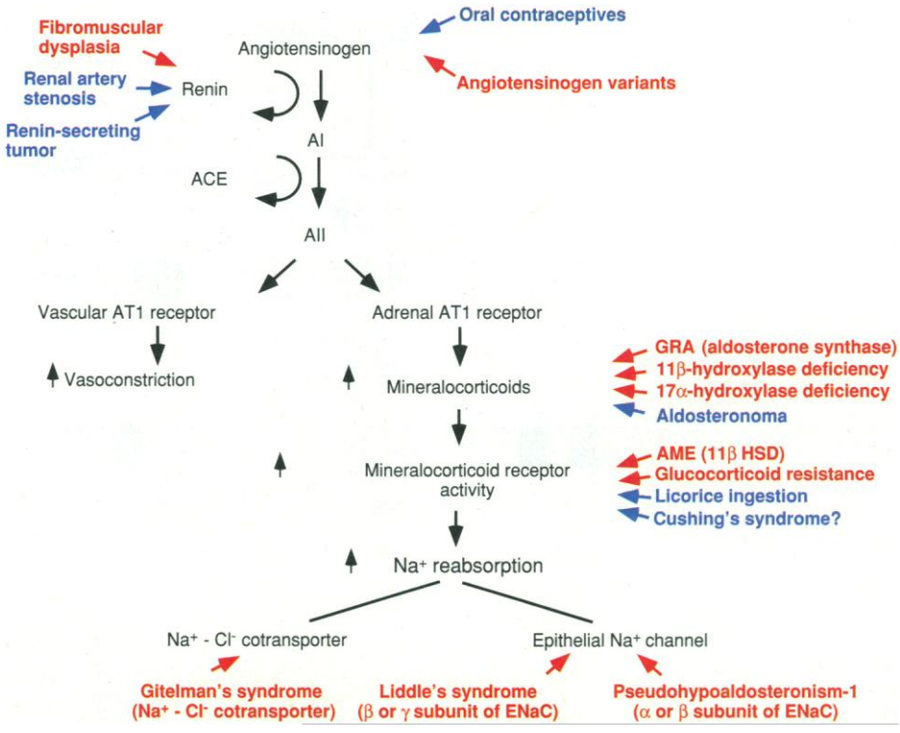

Fig. 1. Blood pressure variation in the renin-angiotensin system. Components of the systemic renin-angiotensin system are shown in black. Angiotensinogen is secreted by hepatocytes. In response to decreased renal perfusion, specialized cells in the kidney secrete renin, which cleaves angiotensinogen to angiotensin I (AI). Angiotensin-converting enzyme (ACE) cleaves AI to form angiotensin II (AII). AII binds to AII receptors of the AT1 class, both in the vasculature and in a specific part of the adrenal cortex, the adrenal glomerulosa, which leads to vasoconstriction and increased secretion of aldosterone, respectively. Aldosterone binds to specific receptors in the kidney, leading to increased renal sodium reabsorption and expanded plasma volume. Genetic disorders that affect blood pressure by altering the activity of this pathway are indicated in red, with accompanying arrows indicating the site in the pathway altered by mutation; genes that are mutated in these disorders are indicated in parentheses. Acquired disorders that alter blood pressure through effects on this pathway are indicated in blue.

enzyme and produces a syndrome similar to AME in individuals consuming large quantities of licorice (*12*). The recent cloning of the kidney-specific isoform of 11β-hydroxysteroid dehydrogenase and the demonstration of mutations in this gene in AME patients that result in loss of enzymatic activity provided definitive evidence that a deficiency in this enzyme underlies this form of hypertension (*14*).

This same mechanism of insufficient 11β–hydroxysteroid dehydrogenase to metabolize cortisol has been proposed to explain the hypertension seen in Cushing's syndrome (*15*) and in glucocorticoid resistance caused by mutations in the glucocorticoid receptor (*16*). For both disorders, there have been reports of elevated cortisol: cortisone ratios and of features of mineralocorticoid effects, including suppression of plasma renin activity and aldosterone levels, low serum potassium levels, and elevated bicarbonate levels.

Liddle's syndrome. Like patients with GRA and AME, patients with Liddle's syndrome present with early and typically moderate-to-severe hypertension (*17*). As in GRA and AME, the pathogenesis of hypertension in Liddle's syndrome entails increased renal reabsorption of salt and water. However, in contrast to these disorders, antagonism of the mineralocorticoid receptor has no effect on blood pressure, and renal transplantation has corrected the defect in a Liddle's syndrome patient (*18*), which suggests that the defect in these patients is intrinsic to the kidney.

Linkage analysis localized the gene causing Liddle's syndrome to a small segment of chromosome 16 (*19*). This segment contained two genes of particular interest—the genes encoding the β and γ subunits of the amiloride-sensitive ENaC. This channel is composed of at least three subunits and normal channel activity requires all three (*20*). (The α subunit gene is located on chromosome 12). Reabsorption of sodium through ENaC is regulated by aldosterone, and normally this regulated step appears to be the major determinant of net renal sodium reabsorption. Examination of these genes in patients with Liddle's syndrome has revealed mutations in genes encoding either the β or γ subunits of the channel (Fig. 2B) (*19*, *21*). These mutations result in deletion of the cytoplasmic COOH-terminus of the subunits or in introduction of amino acid substitutions into a short proline-rich segment of the COOH-terminus. Expression in *Xenopus* oocytes of ENaC containing these mutant subunits produces a markedly increased whole-cell Na^+ current (*22*); this increased activity in vivo leads to increased sodium reabsorption and explains the hypertension seen in affected patients.

Investigation of the mechanism of these gain-of-function mutations has indicated that the short proline-rich segments of the ENaC subunits are the critical targets for activating mutations (*21*, *23*). It appears that these mutations result in an inability to remove active channels from the apical cell surface, a function that is likely to be mediated by another protein binding to the proline-rich segment.

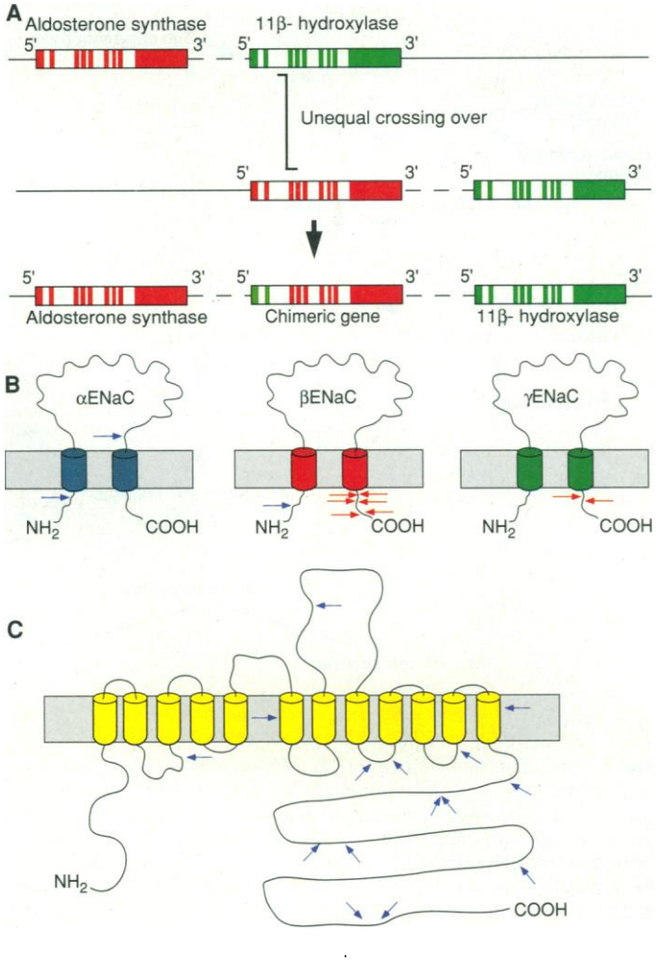

Fig. 2. Examples of mutations that alter blood pressure in humans. (**A**) The chimeric gene duplication causing GRA arises from unequal crossing-over between genes encoding aldosterone synthase and steroid 11β–hydroxylase. Exons of the aldosterone synthase and steroid 11β–hydroxylase genes are shown in red and green, respectively. These genes are linked on chromosome 8 and are separated by 45 kb [reproduced with permission from (*6*) (copyright 1992, Macmillan Magazines, London)]. (**B**) Mutations in subunits of ENaC cause either Liddle's syndrome or PHA-1. Subunits traverse the plasma membrane twice, with intracellular NH_2- and COOH-termini. Red arrows indicate the location of mutations causing Liddle's syndrome; these mutations all cluster in the cytoplasmic COOH-termini of subunits. Blue arrows indicate the location of mutations causing PHA-1. (**C**) Mutations in the thiazide-sensitive Na-Cl cotransporter cause Gitelman's syndrome of hypokalemic alkalosis and salt wasting. The cotransporter is thought to span the plasma membrane 12 times. Blue arrows indicate the location of missense and premature termination codons found in affected patients.

Mendelian Forms of Hypotension

Identification of the molecular basis of several autosomal dominant forms of hypertension has permitted unambiguous identification of mutant gene carriers, allowing the spectrum of blood pressures in gene carriers to be assessed. Some family members who have inherited these mutations have proved to have normal or only minimally elevated blood pressures, which suggests that just as there are alleles that raise blood pressure, there are likely to be alleles that lower blood pressure in the population.

Identification of mutations causing recessive forms of severe hypotension represents one approach to this problem; heterozygous carriers of these same mutations, who will be much more prevalent than their homozygous counterparts, may be protected from development of hypertension. Once relevant mutations are identified, the hypothesis that the heterozygous state lowers blood pressure can be tested. In the first months of 1996, the molecular causes of two inherited forms of hypotension have been reported.

Pseudohypoaldosteronism type 1 (PHA-1). Autosomal recessive PHA-1 is characterized by life-threatening dehydration in the neonatal period, marked hypotension, salt wasting, high serum potassium level, metabolic acidosis, and marked elevation in plasma renin activity and aldosterone levels (*24*). Genetic analysis of affected offspring of consanguineous unions demonstrated linkage of this disease to segments of either chromosome 12 or 16, each containing genes encoding different subunits of ENaC, the same channel mutated in patients with Liddle's syndrome (Fig. 2B) (*25*, *26*). Exam-

ination of the ENaC subunit genes in families with PHA-1 revealed mutations that, in contrast to the activating ENaC mutations that cause Liddle's syndrome, result in loss of ENaC function (*26*). This leads to primary salt wasting from the kidney tubules, accompanied by a secondary defect in secretion of potassium and hydrogen ions. These factors stimulate activity of the renin-angiotensin system; however, salt reabsorption cannot be augmented because the target, ENaC, is defective.

Gitelman's syndrome. This syndrome is an autosomal recessive trait characterized by diverse physiologic and clinical manifestations. Affected subjects have low serum potassium and high serum bicarbonate levels, renal salt wasting, low urinary calcium excretion, low serum magnesium levels, and an activated renin-angiotensin system. Patients with this disorder have low blood pressure and commonly present with neuromuscular abnormalities (*27*). The gene causing Gitelman's syndrome has been mapped to a region of chromosome 16 containing the gene encoding the renal thiazide-sensitive Na-Cl cotransporter (*28*). This ion transporter is present on the apical membrane of renal tubular epithelium in the distal convoluted tubule and mediates electroneutral reabsorption of sodium and chloride (*29*). Patients with Gitelman's syndrome show a diverse array of nonconservative missense mutations, premature termination codons, and splice site mutations in the Na-Cl cotransporter gene that cosegregate with the disease and result in loss of cotransporter function (Fig. 2C) (*28*). This finding demonstrates that the primary defect in these patients is renal salt wasting and that the many other manifestations of the disease must derive from this primary abnormality.

Essential Hypertension

In the vast majority of patients with elevated blood pressure, no underlying cause has been identified, and these patients are classified as having essential hypertension. It seems likely that single gene disorders of blood pressure regulation will cumulatively explain only a small fraction of the total genetic variation in blood pressure in the population, thereby necessitating analysis of traits with less clear-cut modes of inheritance. Among the approaches to this problem are primary linkage strategies using affected (for example, hypertensive) sibling or relative pairs, direct investigation of candidate genes for molecular variants and altered biochemical function in hypertensive or hypotensive populations, and studies contrasting the allele frequencies of genetic markers in particular cases and controls. Use of intermediate phenotypes (that is, traits that identify more genetically homogeneous subgroups of the hypertensive population) can potentially increase analytic power.

Angiotensinogen variants. Of the small number of candidate genes examined (*30*), only the gene encoding angiotensinogen has met relatively stringent criteria supporting its role in the pathogenesis of essential hypertension (*31*). Angiotensinogen is secreted by the liver; sequential cleavage of this protein by renin and angiotensin-converting enzyme produces the active hormone AII, which promotes a rise in blood pressure (Fig. 1). Three independent lines of evidence, replicated in independent samples from Caucasian people in Salt Lake City and Paris, support a role for angiotensinogen variants in essential hypertension: (i) linkage of the angiotensinogen locus with hypertension in hypertensive sibling pairs, (ii) association of specific angiotensinogen variants with hypertension in case-control studies, and (iii) association of these same variants with elevated plasma angiotensinogen levels (*31*).

These findings suggest a model whereby changes in angiotensinogen levels can produce changes in blood pressure. This model has been tested by the engineering of mice carrying from zero to four copies of the normal mouse angiotensinogen gene. The plasma level of angiotensinogen in these animals increases with the number of angiotensinogen genes and is paralleled by graded increases in blood pressure, which strongly supports the model (*32*). At present, the identity of the functional variants in the human angiotensinogen gene, the magnitude of their effects on blood pressure, and their mode of action have not been defined.

An Emerging Pattern

Identification of a number of genes in which mutations contribute to hypertension or hypotension in humans permits an interim assessment of the pathways by which these genes act. It is readily apparent thus far that the genes converge on a final common pathway—the regulation of salt reabsorption in the kidney (Fig. 1). Mutations causing GRA, AME, and Liddle's syndrome all result in constitutively increased renal sodium reabsorption, whereas mutations causing PHA-1 and Gitelman's syndrome reduce blood pressure by diminishing renal salt reabsorption. Angiotensinogen variants also act in this same pathway, probably at least in part through effects on renal salt handling. Patients with steroid 11β–hydroxylase deficiency (*33*) and steroid 17α–hydroxylase deficiency (*34*) commonly develop hypertension as a consequence of overproduction of steroid metabolites with mineralocorticoid activity, and the hypertension in patients with glucocorticoid resistance and Cushing's syndrome is likely to be due in part to saturation of 11β-hydroxysteroid dehydrogenase, which causes cortisol to act as a potent mineralocorticoid.

In addition to these inherited disorders altering blood pressure, the pathogenesis of a number of acquired forms of hypertension has been defined. Virtually all of these disorders are due to altered activity of the renin-angiotensin system and to altered renal handling of salt (Fig. 1). Oral contraceptive–induced hypertension appears to be attributable to increased secretion of angiotensinogen. The hypertension seen in patients with renin-secreting tumors, renal artery stenosis, and a related inherited disorder, fibromuscular dysplasia, is attributable to a marked increase in renin secretion. Aldosterone-producing adenoma causes hypertension through overproduction of mineralocorticoid hormones. The hypertension arising from ingestion of large quantities of natural licorice is attributable to glycyrrhethinic acid, which inhibits 11β-hydroxysteroid dehydrogenase, the enzyme that is genetically defective in patients with AME.

This emergence of the key role of the kidney in blood pressure variation is consistent with a large body of earlier work. Guyton and colleagues have argued that hypertension cannot be sustained without the active participation of the kidney, because elevated renal perfusion pressure leads to salt and water diuresis, returning blood pressure to normal levels; this contention is supported by extensive data from experimental animals (*35*). Several additional lines of evidence also support this model. For example, transplantation of kidneys from genetically normotensive animals into genetically hypertensive recipients can prevent or correct hypertension (*36*). Furthermore, a marked elevation in blood pressure in response to increased dietary salt has been observed in subsets of human (*37*), primate (*38*), and rodent (*39*) populations, again pointing to the importance of inherited variation in renal salt handling.

One can speculate that the evolution of our species in the interior of sub-Saharan Africa, a notoriously salt-poor environment, may have provided strong selective pressure favoring avid renal salt and water retention. Recent experiments changing African primates from their natural low-salt diet to a high-salt diet led to a marked increase in blood pressure, supporting a model in which alleles promoting salt retention may have had adaptive value in our ancestral environment but now contribute to hypertension and its sequelae in

the salt-rich environment of industrialized societies (*38*).

It is important to point out that these observations do not exclude the possibility that genetic effects will be found that operate on blood pressure through other mechanisms. Nonetheless, the finding that the preponderance of known diseases that alter blood pressure in humans act by altering renal salt handling should motivate careful examination of genes whose encoded products mediate or regulate renal sodium reabsorption for a potential causative role in hypertension.

Future Studies

With the completion of dense genetic maps of the human genome (*40*), linkage studies of complex traits such as essential hypertension can be performed for comprehensive mapping of genes that contribute to blood pressure variation. The utility of this approach has been demonstrated for another complex trait, type I or insulin-dependent diabetes mellitus (*41*). In addition, approaches exploiting linkage disequilibrium resulting from founder mutations can be applied in either primary mapping studies (*42*) or in refinement of the location of disease genes (*43*). It is anticipated that gene identification will be facilitated by detailed knowledge of the physiology of blood pressure regulation, both by suggesting candidate genes in linked chromosome regions and by suggesting testable hypotheses of mechanisms by which identified mutations might alter blood pressure. Continued progress in identification and localization of genes on maps of the human genome will probably play a key role in the identification of candidate genes in linked regions. Once identified, the physiologic consequences of identified mutations can be assessed at the biochemical and clinical levels, providing new opportunities to assess responses to therapy among patients harboring specific genetic variants as well as factors such as gene-gene and gene-environment interaction.

As in humans, blood pressure variation among experimental animals has been found, and a number of inbred strains with contrasting blood pressures have been developed. Genetic approaches to identification of underlying genes influencing blood pressure can be pursued in these animals, with the advantage that desired matings can be produced and environmental factors can be rigorously controlled (*44*). Genes implicated in hypertension in experimental animals are plausible candidates for roles in human hypertension and may also identify novel targets for therapeutic intervention. The engineering of new animal models based on mutations identified in human studies will also prove invaluable, providing novel opportunities to examine the effects of particular mutations in different defined environments and to explore the physiologic consequences of different combinations of mutant genes (*45*).

In parallel to investigation of genes determining blood pressure variation, similar investigation of genetic determinants of clinical outcomes related to hypertension may be productive. Stroke, myocardial infarction, and end-stage renal disease all show inherited susceptibility not accounted for by known susceptibility alleles. Identification of these susceptibility genes by approaches similar to those taken to hypertension has the potential to identify new targets for early therapeutic intervention in order to prevent early morbidity and mortality from these diseases.

Success in identification of genes conferring susceptibility to hypertension and its clinical sequelae is expected to provide new insights into disease pathophysiology and lead to development of genetic tests of high accuracy, permitting identification of subjects with specific inherited susceptibility. These insights may permit intervention in preclinical stages with therapies tailored to underlying primary abnormalities, improving efficacy of treatment and reducing morbidity and mortality from these diseases.

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