Review

Genetic Analysis in Human Hypertension

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Hypertension is considered to be a complex trait to which genetic, environmental, and demographic factors contribute interactively. Recently, molecular genetic studies have achieved remarkable success in the elucidation of causative mutations in several Mendelian hypertensive disorders in which single nucleotide polymorphisms (SNPs) disrupt the function of single genes, thereby leading to unambiguous phenotypes. It seems unlikely, however, that such a simple base-substitution is the primary mechanism in cases of essential hypertension, even if SNPs modify the relevant gene function to some extent. Despite the enormous efforts made to date, no consistent association between any of the candidate genes and essential hypertension has been established. One plausible explanation is that because individual genes play a modest role in the pathogenesis of hypertension, confounding variables, whether individual (sex, ethnic origin, etc.) or environmental, may decrease the chance of identifying a causative relation between the genes and hypertension, depending on the populations studied. Several approaches can be proposed to overcome this problem, including long-term follow-up of clinical events collected to attain sufficient phenotypic information and statistical power. With the recent advances in high-throughput genotyping techniques and bioinformatic strategies, it has become possible to perform even SNP-based genome-wide screening. At present, however, the need for identification of susceptibility genes for hypertension still poses a great and unanswered challenge. Nonetheless, we believe that a precise understanding of the manner in which genetic variations affect hypertension can be achieved, and that clarification of the associated phenotypes will lead to the development of effective preventive and treatment strategies. (*Hypertens Res* 2002; 25: 319–327)

Key Words: genetics, polymorphism, association, linkage, quantitative trait loci

Introduction

Essential hypertension is a multifactorial trait involving interactions among genetic, environmental, and demographic factors. While hypertension itself does not always lead to apparent clinical symptoms, it represents a major health burden due to its association with an increased risk of certain vascular disorders, such as myocardial infarction and stroke. A number of classes of antihypertensive drugs developed to date have shown satisfactory, therapeutic effects in some but not all patients. Under the circumstances, studies of molecular genetics are expected to play a key role in establishing more efficient and more radical treatment in hypertension.

Recently, remarkable progress has been achieved in clari-

fying the molecular basis of Mendelian hypertensive disorders (1). Causative genes and chromosomal fragments harboring disease susceptibility genes have been identified for glucocorticoid-remediable aldosteronism (2), Liddle's syndrome (3, 4), the syndrome of apparent mineralocorticoid excess (5) and so on (6, 7). On the other hand, although extensive searches have been performed for essential hypertension, most of the results have been inconclusive. Several explanations may be proposed for the difficulties in detecting susceptibility genes for essential hypertension (8). First of all, according to biometric studies, the relative contribution of genetic factors to blood pressure (BP) variation is estimated to be rather modest as compared to other multifactorial traits (9). In addition, the genetic complexity underlying essential hypertension should be taken into consideration (10).

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Fig. 1. Progress in molecular genetics of hypertension in rats and in humans.

Not only can numerous physiological pathways regulate BP by constituting a close network within the body, but also a variety of organs, *e.g.*, the brain, heart, kidneys, adrenal glands, and blood vessels, are involved in individual pathways. To overcome the substantial genetic complexity, extensive studies have been performed using model organisms of hypertension, particularly inbred hypertensive rat strains, in parallel with studies in humans (Fig. 1).

In this article, I review experimental strategies and recent advances in genetic analysis in human hypertension.

Methodological Basis of Genetic Analysis in Hypertension

Key Concepts

Epidemiological (or classical genetics) studies have indicated that BP is a multifactorial trait with about 30% to 60% of the phenotypic variation being attributed to genetic factors (11). During the last several years, innovative research techniques have been devised and have enabled us to investigate biological mechanisms involved in hypertension at the cellular and molecular levels. Nevertheless, the key question of what is genetically impaired in essential hypertension remains to be answered.

A frequently proposed hypothesis in hypertension genetics is that individual mutations or functional polymorphisms occurred somewhere in the world in an individual or individuals at some point in human history, and then these mutations, along with the neighboring loci on the “affected” chromosomes, were retained as an ancestral fragment over many generations. In this way, the frequency of the linked genes exceeds or is in disequilibrium with that predicted by multiplying together their individual frequencies. This phenomenon is known as linkage disequilibrium. Most currently applicable molecular genetic strategies for determining multifactorial traits exploit this concept by using genetic markers

Fig. 2. Frequently proposed hypothesis concerning the molecular basis of essential hypertension. Blood pressure (BP) is assumed to have a quasi-unimodal distribution in the general population. The higher the BP values of a certain individual are, the greater the chance that should exist for him or her to possess a disease-type (variant-type) allele of a given susceptibility gene. Also, when focusing on individual subjects rather than the population at large, a greater number of susceptibility genes are likely to be impaired in the hypertensive subject than in the normotensive subject.

located close enough to a causative mutation of the gene to be in linkage disequilibrium, and thereby identifying chromosomal fragments harboring the gene in question.

As a simple example, let us focus on a situation in which each susceptibility gene exerts substantial genetic effects on BP regulation (Fig. 2). In the general population, BP is assumed to have a quasi-unimodal distribution. The higher the BP values of a certain individual are, the greater the chance should be that he or she possesses a disease-type allele of a given susceptibility gene, and the greater the number of susceptibility genes that are likely to be impaired.

Table 1. Comparison between Two Principal Strategies of Molecular Genetics in Human Hypertension

	Association study	Linkage analysis
Sample collection	unrelated individuals in case-control study panels	2–3 generations of affected families or sibling-pairs
Statistical power (GRR detectable using 1,000 samples)	relatively large (GRR > 1.5)	poor (GRR > 4.0)
Liability to false positive results	relatively high	relatively low
Target genes	frequently focus on candidate genes	not necessarily select candidate genes
Chromosome fragment size in which linkage is supposed to be detectable	< 3 kb– > 50 kb	10–20 cM (> 10 Mb)
Genetic markers required to perform genome-wide searches	> 100,000 SNPs	300–400 microsatellite markers

SNPs, single nucleotide polymorphisms; GRR, genotype relative risk.

General Strategies

Two principal strategies—association study and linkage analysis—have been used to investigate the genetic basis of essential hypertension. These two strategies are not mutually exclusive, but can be merged into a single analytical method, such as transmission/disequilibrium test (TDT) (12). Each has advantages and disadvantages depending on the situations tested, as summarized in Table 1. For example, because the association *i.e.*, case-control study (which tests for different allele or genotype frequencies between case and control populations) allows the use of unrelated individuals, it is easier to collect a large set of samples using this paradigm than using a pedigree-based linkage analysis. In general, a case-control study has greater statistical power than a linkage analysis, but it is also more liable to show false positive results. From a methodological point of view, genetic studies can be categorized into candidate gene approaches and genome-wide screens. Association studies are adequate for testing candidate genes, or narrowing down to a particular gene once a region of linkage has been detected. However, the current method of choice for identifying genetic effects independently of the knowledge of *a priori* candidates is genome-wide linkage analysis.

Four main strategies have been used to examine linkage of genes or chromosome regions to hypertension (or BP levels), depending on the method used to select the loci to be tested, and the type of families to be studied (Table 2): 1) studies of Mendelian forms of hypertension; 2) testing of candidate genes chosen on the basis of their known biochemical or physiological function; 3) investigation of chromosome regions homologous to those that segregate with BP in animal models, or regions harboring particular genes that show linkage in animal models; and 4) systematic genome-wide searches for linkage (or linkage disequilibrium). Notable findings attained by each strategy will be outlined later.

Statistical Significance and Study Power

In general two-point linkage studies, an LOD score of 3 is

classically accepted as statistically significant. However, multiple testing (*i.e.*, for different markers), as in the context of a genome-wide screen, usually occurs without the prior knowledge of the number of genes involved in the disease, so that the significance level to declare linkage is problematic. Lander and Kruglyak (13) have defined guidelines to account for the multiple testing and have estimated that an LOD score of 3.6 (nominal *p*-value of 2×10^{-5}) would correspond to a significance level of 0.05 (*i.e.*, this value of LOD score or higher is expected to be found by chance one in every 20 genome-screens performed). In contrast with the genome-wide significance level, the point-wise significance level, as in the context of a candidate gene approach, is the probability that one would encounter an extreme deviation at a specific locus (or a limited number of loci) by chance. Even though the latter concerns a single test of the null hypothesis of no linkage and/or no association at a time, a number of loci (or candidate genes) are repeatedly evaluated on the same set of samples, and multiple testing is unavoidable. Therefore, to control the possible inflation of the “type I” error, an appropriate adjustment (*e.g.*, Bonferroni correction) should be applied to the point-wise significance level.

The strength of genetic effects in hypertension needs to be considered in terms of study power. A parameter frequently used for evaluating genetic effects in multifactorial diseases is “ λ s”: the risk of developing a trait or disease in siblings of affected probands relative to that in the general population (14). The λ s for hypertension seems to be relatively low, ~ 4 , compared to those for other common multifactorial diseases: the λ s for multiple sclerosis is 20 to 40, that for Crohn’s disease is 25 to 35, and that for insulin-dependent diabetes mellitus is 15 (9, 15). Suppose that a single “major” susceptibility gene would result in a 4-fold increase in the risk of developing hypertension in an additive inheritance manner (*i.e.*, the genotypic relative risk is 2.0); in this case, more than 3,000 sib-pairs or fewer than 500 case-control pairs would be required for an affected sib-pair linkage analysis and a case-control study, respectively, to provide sufficient statistical power (16). A number of investigators have performed power calculations to evaluate the probability of detecting

Table 2. General Strategies to Investigate Genetic Basis of Hypertension

1. Studies of Mendelian forms of hypertension (mutated genes listed in the right side of the arrows)
Glucocorticoid-remediable aldosteronism (GRA) aldosterone synthase (<i>CYP11B2</i>) gene
11 β -hydroxylase deficiency 11 β -hydroxylase (<i>CYP11B1</i>) gene
Syndrome of apparent mineralocorticoid excess (AME) 11 β -hydroxysteroid dehydrogenase kidney-type (<i>HSD11B2</i>) gene
Hypertension exacerbated by pregnancy mineralocorticoid receptor (<i>MR</i>) gene
Liddle's syndrome β and γ subunits of the epithelial sodium channel (<i>ENaC</i>) gene
2. Candidate genes chosen on the basis of known biochemical or physiological function
1) Renin-angiotensin system
Angiotensinogen (<i>AGT</i>), renin (<i>Ren</i>), angiotensin converting enzyme (<i>ACE</i>), angiotensin II type 1 and type 2 receptors
2) Sympathetic nervous system
β 1 and β 2 adrenergic receptors
3) Ion transports
G-protein β 3 subunit (<i>GNB3</i>)
4) Others
Vasoactive peptides, <i>e.g.</i> , eNOS, ANP, ADM
Components involved in insulin resistance or metabolic function, <i>e.g.</i> , lipoproteins
3. Candidate genes or chromosome regions reported in rat hypertension
SHR: SA on rat chromosome 1
Dahl salt sensitive rat: 11 β -hydroxylase on rat chromosome 7
Milan hypertensive strain: α -adducin on rat chromosome 14
4. Positional candidate genes (mostly) in chromosome regions implied by genome-wide screens
Chromosome 3: Angiotensin II type 1 receptor
Chromosome 5: β 2 adrenergic receptor
Chromosome 8: lipoprotein lipase
Chromosome 17: ACE

linkage and association for genetic effects of a given strength (as measured by λ s).

Notable Findings Reported in Human Hypertension

Mendelian Forms of Hypertension

Genes involved in monogenic disorders are much easier to map than those involved in multifactorial traits. Consequently, one way to reduce, to some extent, the genetic complexity of hypertension is to explore linkage in extended families which display a quasi-Mendelian type of inheritance. Several rare syndromes that are associated with hypertension and that are influenced by one or more mutations have been described (Fig. 3). Interestingly, most of the causative genes identified for Mendelian forms of hypertension have turned out to be involved in the renin-angiotensin (R-A) system or components downstream. These findings support the possible etiological importance of the R-A system in hypertension.

Despite the rarity of these syndromes, the identification of the corresponding genes may help to clarify the genetics of essential hypertension for two reasons: "milder" variants in these same genes may be relatively frequent in the general

population and contribute to common, essential hypertension; and similar physiologic pathways may be relevant to both rare and common forms of hypertension. For example, because gain-of-function mutations in the β or γ subunit of the epithelial sodium channel (*ENaC*) gene result in Liddle's syndrome (3, 4), this channel has been frequently investigated in a number of different populations. Of note is the fact that the T594M polymorphism of the β subunit was associated with a disease phenotype of essential hypertension in black residents in London (17). However, this association was not replicated in black Americans (18), and the 594M variant itself was rarely seen in other ethnic groups (19).

On the other hand, it is conceivable that some patients currently diagnosed with essential hypertension will be recategorized into a new class of secondary hypertension based on the individual genotype information. One gene that has led to such reassignment is the mineralocorticoid receptor (*MR*) gene. While loss-of-function mutations of *MR* were known to cause pseudohypoaldosteronism type I, a disease characterized by salt wasting and hypotension (20), Geller *et al.* (6) recently identified a gain-of-function mutation, S810L, which is responsible for early-onset (severe) hypertension exacerbated in pregnancy. Normally, activation of *MR* by the steroid hormone aldosterone raises renal salt reabsorption in the distal nephron. The mutation, S810L, results in consti-

Fig. 3. Causative genes identified for Mendelian forms of hypertension.

tive MR activity and alters receptor specificity, with progesterone, normally an MR antagonist, becoming a potent agonist. In an attempt to determine the relevance of this MR mutation to severe hypertension, we screened 247 Japanese subjects with severe hypertension, but did not find any relevant mutations (19).

Angiotensinogen

In 1992, Jeunemaitre *et al.* (21) reported significant evidence for linkage and association of the angiotensinogen (*AGT*) gene to hypertension. This finding heralded a spate of “complex” trait investigations into hypertension genetics in humans. In addition to the known physiological importance of *AGT*, it seemed fairly reasonable that *AGT* polymorphisms might not only be associated with plasma concentrations of *AGT*, but might also increase the eventual risk of developing hypertension. Several lines of evidence have supported the etiological link between *AGT* and hypertension; for example, in transgenic mice, the increasing number of *AGT* alleles was shown to be correlated with blood pressure elevation (22). A “functional” polymorphism, G-6A, which was in complete linkage disequilibrium with the originally reported M235T polymorphism, was found in the core promoter region of *AGT* (23). While a number of investigators attempted

to reproduce the original findings of linkage and association at the *AGT* locus, the results have not been always concordant among the studies and have provoked heated arguments (24, 25). Moreover, female-specific association was observed in a large population-based study, the Copenhagen City Heart Study (26). Thus, it remains unknown whether the lack of replication between studies may reflect ethnic variations, differences in diagnostic criteria, family ascertainment, other confounding variables, and/or the modest genetic contribution attributable to this locus.

Angiotensin-I Converting Enzyme and Human Chromosome 17

The angiotensin converting enzyme (*ACE*) locus has been shown to cosegregate with blood pressure in several rat crosses (27), but it remains unclear whether *ACE* itself or a nearby locus (or loci) actually confers susceptibility to rat hypertension. In humans, convincing evidence of linkage and association of the *ACE* locus with serum *ACE* levels have been demonstrated (28), whereas conflicting results have been published regarding the association of *ACE* variants and hypertension (29, 30). Three recent studies, two in whites (31, 32) and one in the Japanese (33), independently reported some evidence of linkage between the *ACE* locus

Table 3. Genetic Analysis of Hypertension in Inbred Rats and Humans

	Inbred rats	Humans
Expected number of susceptibility genes	several genes with major genetic effects	unknown
Heritability of hypertension	~60%	30–40%
Environmental factors	controlable	uncontrolable
Onset of hypertension	early age	advanced age
Genetic homogeneity	high (inbred)	low (outbred)
Reproducibility of BP measurements	relatively good	relatively poor
Analytical approaches	QTL mapping in experimental crosses Development of congenic strains	Linkage analysis (<i>e.g.</i> , affected sib-pair) Association study (unrelated subjects)

QTL, quantitative trait loci.

and hypertension in men but not in women. However, it was also shown that the relation between the *ACE* locus and hypertension was not consistently seen in men but changeable dependent on the age and body weight of the participants (34).

The region of linkage to BP variation near *ACE* in the rat is broad, and genes other than (or in addition to) *ACE* could be responsible for the genetic effect on BP variation. Julier *et al.* (35) have addressed this issue by undertaking a detailed investigation of the homologous region on human chromosome 17 and familial essential hypertension using a total of 518 sibling pairs. The region of significant linkage included the *ACE* locus, but the maximum evidence of linkage was observed at markers located approximately 18 cM proximal to this locus. Linkage to the overlapping region was replicated in another study of familial essential hypertension (36) and in some pedigrees this linkage was found to segregate a dominantly inherited form of hypertension, pseudohypoaldosteronism type II (or Gordon's syndrome) (7). The genes underlying one or both of these traits could be homologous to the gene or genes involved in the BP regulation on rat chromosome 10 represented by the *ACE* locus.

G-Protein 3 Subunit

Enhanced signal transduction *via* pertussis toxin-sensitive G-proteins has been demonstrated in immortalized lymphoblasts from hypertensive patients (37) and this transduction may underlie the sodium-proton transport abnormality observed in blood cells of a subgroup of hypertensive subjects (38). In the search for structural changes in the α , β , and γ subunits of heterotrimeric G-proteins, Siffert *et al.* (39) have recently shown that a C825T polymorphism of the gene encoding the G-protein β 3 subunit is significantly associated with essential hypertension in a white population. This polymorphism does not in itself cause amino acid substitution, but the disease-type (825T) allele was also associated with the occurrence of alternative splicing, which caused the loss of 41 amino acids within highly conserved repeating units of the gene. Although a number of studies have attempted to replicate this association in a variety of populations, conflict-

ing results have been reported (40, 41).

Chromosomal Regions (or Candidate Genes) Homologous to BP-Linked Regions in Rats

Studies of animal models of hypertension, especially inbred hypertensive rats, circumvent many of the problems encountered in human studies (Table 3). BP measurement can be done repeatedly under more controlled conditions and may be more reproducible than in humans. Environmental "noise" and genetic heterogeneity can be reduced considerably, since animals can be raised in the same environmental conditions. Studies of gene interactions can be performed in these animal models, and the use of congenic animals constitutes a powerful tool to isolate and test specific chromosome regions in various genetic backgrounds. In addition, genes predisposing to hypertension in animal models may also be involved in the etiology of human hypertension, and hence the regions of homology, or more directly the genes implicated in animal models, can be considered candidate regions or genes to be explored in the human disease.

Among such candidate genes, the α -adducin gene has drawn substantial attention. Amino acid variations in the α - and β -subunits of adducin, a membrane skeleton protein, have been shown to be associated with faster ion transport in the Milan hypertensive strain compared to the Milan normotensive strain (42). In humans, Cusi *et al.* (43) found a segregation at microsatellite markers near the α -adducin gene as well as a suggestion of association of the 460Trp variant with both hypertension and salt sensitivity. Although these independent lines of evidence are compelling, replication studies in other populations have not necessarily confirmed the implication of α -adducin in human hypertension.

Apart from α -adducin, causative genes remain to be identified in most quantitative trait loci (QTLs) for BP reported in rats to date. Since model organisms have been used, there has been debate about the utility of these models in hypertension genetics. Nevertheless, under the current circumstances in which little is known about the genetic basis of human hypertension, chromosomal regions homologous to BP-linked regions in rats can be regarded as potential candi-

date hypertension loci in humans. Along these lines, Jacob and associates (44) proposed a "comparative genomics strategy" and predicted 26 chromosomal regions of the human genome that should be prioritized in searches for single nucleotide polymorphisms (SNPs) and linkage disequilibrium testing.

Genome-Wide Linkage Search

A number of recent genome screens have provided evidence suggesting linkage in more than 10 chromosomal regions, among which several positional candidate genes were inferred; *e.g.*, the angiotensin II type 1 receptor gene on chromosome 3 (45), the β_2 -adrenergic receptor (*ADRB2*) gene on chromosome 5 (46), and the lipoprotein lipase gene on chromosome 8 (47). In most cases, the linked regions were not overlapped between the studies, though reproduction of the findings in a different ethnic population is generally considered to be important. In reality, polymorphisms (of a certain gene) could be associated with a phenotype in one ethnic population but not in another, and hence different genes may predispose to the phenotype of hypertension in different populations.

The most difficult part of the reverse genetic method, *i.e.*, the genome-wide screen, would be to provide convincing evidence for the disease causality of one specific gene (but not the other neighboring genes). Due to the difficulty of collecting sufficient samples for a large-scale pedigree-based study, investigators have tended to focus on positional candidate gene approaches to finding responsible genes in the regions showing some evidence of linkage. In general, these approaches have been conducted by an association study (or a test of linkage disequilibrium) and have examined the relationship between a disease phenotype and the gene in question with the remainder of the genome being more or less ignored. With recent advances in high-throughput genotyping techniques and bioinformatic strategies, it has become possible to perform even genome-wide SNP-based screening, thereby enabling us to simultaneously evaluate multiple gene loci in consideration of epistasis.

2 Adrenergic Receptor Gene and Human Chromosome 5q

The long arm of human chromosome 5 contains a cluster of genes encoding the β_2 and α_{1B} adrenergic receptors and the dopamine receptor type 1A, any of which may be involved in BP regulation. Krushkal *et al.* (48) have recently investigated this region by discordant sib-pair linkage analysis as well as by the transmission/disequilibrium test, both of which implied the presence of susceptibility gene(s) in this region on chromosome 5q. On the basis of its physiological importance and biological credibility, the *ADRB2* gene has been noted as a promising candidate gene. Thus, a number of association studies have repeatedly tested a few *ADRB2* polymorphisms which are known to be "functional" in ex-

periments *in vitro*, but contradictory results have been observed in different populations (49).

In conclusion, with the recent progress in molecular genetics, there has been some progress made towards understanding of the genetics of hypertension. Studies of Mendelian forms of hypertension have led to the identification, or mapping, of several genes. The extent of the implication of some of these genes in essential hypertension remains to be established. Although candidate gene studies in humans have failed to detect major genetic effects on hypertension, suggestion of linkage, as well as association, have been shown in some studies at the *AGT* and *ACE* loci, and may correspond to a weak genetic contribution of these genes. Genome-wide screens have revealed a number of chromosomal regions which may harbor QTLs for hypertension. Because of the complex nature of the hypertension phenotype, large-scale studies will be required to definitively establish the role of the specific chromosomal regions or genes reported so far, or in the future, as well as to explore the effect of confounding variables, whether individual (sex, ethnic origin, *etc.*) or environmental. Also, given the modest genetic impact generally attributable to single loci, prospective epidemiological evaluation in the general population will also be required. The combination of genetic methods (such as linkage and association analyses) and multiple independent approaches (in animal models or Mendelian forms of hypertension) is the strategy of choice to clarify the disease relevance of a susceptibility gene.

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