Gene Mapping in Experimental Hypertension

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ABSTRACT
In the rat, the results of genetic linkage studies by "candidate" gene or "positional mapping" approaches have suggested that DNA sequences that regulate blood pressure may be located in the vicinity of the kallikrein gene family on chromosome 1, the gene for angiotensin-converting enzyme on chromosome 10, the renin gene on chromosome 13, and the major histocompatibility complex on chromosome 20. Some studies have also suggested that blood pressure regulatory genes may be located on the sex chromosomes. Pending the results of confirmatory studies, these experiments should be interpreted with caution. However, with confirmation of these studies, it should be possible to create a variety of new animal models that will provide excellent opportunities for investigating the molecular, biochemical, and physiologic determinants of high blood pressure. In addition, in genetic studies in humans with essential hypertension, it may be worthwhile to target chromosome regions that are homologous to those implicated in linkage studies of hypertension in rodents. By narrowing the focus on selected areas of the genome, experimental linkage studies in the rat may also be used to guide the detailed molecular approaches ultimately required to identify the specific DNA sequence alterations that give rise to increased blood pressure.

Key Words: Spontaneously hypertensive rat, blood pressure, polymorphism, linkage analysis, renin

Essential hypertension is believed to be a heterogeneous disorder in which a variety of environmental and genetic factors interact to increase blood pressure. Although substantial progress has been made in characterizing the environmental factors that affect blood pressure, much less progress has been made in identifying the genetic determinants of hypertension. Because genetic studies of multifactorial disorders in humans remain highly problematic, some investigators have chosen to conduct linkage studies in rodent models of hypertension. It is hoped that the identification of genes contributing to increased blood pressure in animals might shed light on the pathogenesis of essential hypertension in humans.

The spontaneously hypertensive rat (SHR) and the Dahl salt-sensitive rat are the most widely studied animal models of human hypertension. The SHR and Dahl SS/Jr strains are particularly amenable to genetic studies because they are highly inbred and constitute discrete genetic forms of hypertension in which just a few major loci may be determining the increased blood pressure. In animal models in which multiple minor genes determine the increased blood pressure, it will be much more difficult to dissect out the individual loci contributing to the hypertension. During the experimental development of the SHR and Dahl strains, severe hypertension became fixed within several generations of brother × sister mating (1,2). This observation suggests that the hypertension phenotype in these models is largely determined by just a few major genes. In genetic crosses involving SHR and inbred normotensive Lewis or Wistar-Kyoto (WKY) rats, blood pressure has been found to fit an additive-dominance model of inheritance in which alleles decreasing blood pressure are partially dominant (3).

Over the past three decades, many investigators have attempted to identify genetic determinants of hypertension by systematically comparing the SHR and Dahl salt-sensitive strains with their respective normotensive "controls," the WKY rat and the Dahl salt-resistant rat. As emphasized by Rapp, however, such interstrain comparisons are of limited value for identifying the pathogenetic determinants of hypertension (4). These hypertensive and normotensive strains differ with respect to multiple genes, not just those affecting blood pressure. In fact, most of the physiologic and biochemical differences observed between these hypertensive and normotensive strains are probably not causally related to their differences in blood pressure (5–7). Many such biochemical and physiologic differences can be explained by genetic drift and the random fixation of alleles at loci that are not involved in the regulation of blood pressure. Furthermore, it is possible that some of the physio-
logic and biochemical differences observed between the hypertensive and normotensive rats could be secondary to their differences in blood pressure. Attempts to circumvent this problem by studying "pre-hypertensive" rats may not be valid because blood pressure in the hypertensive strains may be higher than that in the normotensive strains even at birth (and perhaps in utero) (8–10).

GENERAL APPROACH TO THE IDENTIFICATION OF GENES REGULATING BLOOD PRESSURE

Approximately 10 yr ago, Rapp published a paradigm for searching for primary genetic causes of spontaneous hypertension (11). In segregating populations derived from crosses of an inbred hypertensive strain and an inbred normotensive strain, he proposed studying the relationship between blood pressure and the inheritance of various genetic markers. If the blood pressure of progeny that inherited a certain genetic marker from the hypertensive progenitor strain was greater than that of progeny that inherited the corresponding genetic marker from the normotensive progenitor strain, this would suggest the presence of a blood pressure regulatory locus in the vicinity of the genetic marker (Figure 1). Because increased blood pressure may be determined by multiple genes, a single genetic marker would be expected to account for only a portion of the difference in blood pressure between the hypertensive and normotensive progenitor strains.

With the advent of restriction fragment length polymorphisms (RFLP) and polymerase chain reaction methods for detecting DNA sequence variation (12), a vast array of molecular genetic markers have become available for such linkage studies of hypertension. In addition, the availability of user-friendly computer programs has greatly facilitated the process of data analysis, particularly for studies in which multiple markers are analyzed simultaneously (13, 14). Thus, it may soon be possible to identify many if not all of the chromosome regions that contain genes important to the pathogenesis of rodent models of spontaneous hypertension.

CANDIDATE GENE APPROACH TO EXPERIMENTAL HYPERTENSION: THE EXAMPLE OF RENIN

Using the candidate gene approach, Rapp and colleagues were the first to successfully employ DNA markers in linkage studies of hypertension (15). In an F2 population derived from Dahl salt-sensitive and Dahl salt-resistant rats, these investigators found that the blood pressure of rats that inherited a RFLP marking the renin allele of the salt-sensitive strain was greater than that of rats that inherited the corresponding RFLP marking the renin allele of the salt-resistant strain (15). Such a finding may be interpreted in several different ways: (1) the cosegregation of renin gene polymorphisms and blood pres-
sure could be a false-positive result because of chance or experimental error; (2) sequence variation in the renin gene could affect blood pressure and contribute to the pathogenesis of hypertension; or (3) the renin RFLP could be serving as a marker for a blood pressure regulatory sequence linked to the renin locus (the linked sequence could be functionally unrelated to renin or could control renin gene expression).

To minimize the likelihood of false-positive linkage results, stringent statistical criteria (e.g., analysis of variance significance values on the order of 0.001 or even lower) should be considered when reviewing genetic studies of hypertension (14). It must be recognized, however, that appropriate critical thresholds for candidate gene studies of multigenic traits like blood pressure have never been clearly defined. Therefore, particular emphasis should be placed on replicating those studies in which candidate gene polymorphisms appear to cosegregate with blood pressure. In Dahl rats, consistent results have been obtained in both F2 and backcross linkage studies of renin gene polymorphisms and blood pressure (16). The possibility of a false-positive result in this circumstance would seem unlikely.

Recently, in an F2 population derived from the SHR and the normotensive Lewis strain, we also found evidence suggesting that a blood pressure regulatory locus may exist in the vicinity of the renin gene (17). When setting up the classical crosses, we deliberately used Lewis rats instead of the more commonly used WKY "controls" because: (1) at the time of breeding, commercially available rats designated "WKY" did not appear to constitute a single inbred strain (6,18) and (2) the possibility of detecting polymorphisms between SHR and Lewis rats appeared to be greater than between SHR and WKY rats (SHR being more distantly related to Lewis rats than to WKY rats). The blood pressure in the F2 rats that inherited the SHR renin allele tended to be greater than that in the F2 rats that inherited only the Lewis renin allele (17). In a separate study in a large set of recombinant inbred strains derived from the SHR and the normotensive Brown Norway rat, Pravenec et al. also found evidence suggesting that a blood pressure regulatory sequence may be located in or near the renin gene (19).

In contrast to the results in Dahl rats and in SHR, F2 studies involving a stroke-prone strain of SHR and a European strain of WKY did not suggest an association between blood pressure and sequence variation in the renin gene (20). It is possible that the renin alleles of some strains of SHR may have a greater capacity to increase blood pressure than do those of some Lewis or Brown-Norway strains, but not those of certain Wistar-Kyoto strains. It should also be recognized that the genetic backgrounds of the different normotensive strains used to establish the segregating populations may influence the capacity of a given renin allele to affect blood pressure. In one genetic background, a particular allele may confer an increase in blood pressure, whereas in another genetic background, the same allele may have little or no effect on blood pressure (16). Similarly, the experimental environment (e.g., diet salt content, stress level, etc.) should also be considered when the capacity of a given allele to affect blood pressure is being evaluated. Taken together, the studies in the SHR along with those in the Dahl strain would appear to indicate that in the rat, a variety of different renin alleles exist and that some of these alleles (but not all) may have different capacities to affect blood pressure.

Given that the renin-angiotensin system is an important determinant of sodium balance and vascular resistance, it seems reasonable to suspect that the renin gene itself is the blood pressure regulatory locus detected by these linkage studies. As previously mentioned, however, it is also possible that the renin RFLP is simply marking a blood pressure regulatory sequence linked to the renin locus. We recently used a panel of rat × mouse somatic cell hybrids to map the rat renin gene to chromosome 13 (19). This region of chromosome 13 appears to carry other candidate genes for hypertension, including the gene that encodes the α-2 subunit of the Na⁺, K⁺-ATPase (unpublished observations).

It should be possible to further localize the blood pressure regulatory gene that maps in the vicinity of the renin locus by creating new strains that are genetically identical except in selected regions of chromosome 13 (strains that are genetically identical except for a single chromosome segment are said to be congenic). For example, one could first compare the blood pressure of the Dahl salt-sensitive strain with the blood pressure of a nearly identical (congenic) Dahl strain that carries a segment of chromosome 13 from the Dahl salt-resistant strain. If these congenic strains were found to exhibit a difference in blood pressure, this would confirm the presence of a blood pressure regulatory locus on that segment of chromosome 13 (the only region of the genome for which the strains differ). By constructing several congenic Dahl salt-sensitive strains, each carrying different segments of chromosome 13 from the Dahl salt-resistant strain, one could further localize the position of the blood pressure regulatory locus on that chromosome.

Backcross breeding and genomic selection techniques can be used to transfer defined chromosome segments from one strain onto the genetic background of another strain (21,22). In each breeding cycle, one can selectively transfer specific chromosome segments by genotyping the backcross progeny for polymorphic markers that map along the target chromosome; polymorphic markers on other chro-
mosomes can be used to select against the remainder of the genome. In preliminary studies, we have found evidence suggesting that congenic Dahl strains that differ in the vicinity of the renin locus exhibit a difference in blood pressure (23; unpublished observations). Additional congenic strains are being developed to further localize the blood pressure regulatory gene that maps near renin on chromosome 13.

Although congenic strains can be used to narrow down the chromosome position of a blood pressure regulatory locus, they cannot be used for fine mapping purposes. At best, the resolution of linkage approaches for mapping blood pressure regulatory genes may be on the order of a few centimorgans. (A centimorgan is a measure of genetic distance that is determined by estimating the frequency of crossing over between two genes. In terms of physical distance, one centimorgan corresponds to approximately 1 million base pairs of DNA.) Thus, once a blood pressure regulatory locus is mapped to a segment of chromosome that is a few centimorgans long (i.e., once the gene is mapped within a few million base pairs of DNA), further localization of the gene will still be a daunting task. However, the identification of specific chromosome regions that contain genes regulating blood pressure will provide direction for selecting appropriate candidate genes for further study. Ultimately, gene targeting techniques such as homologous recombination will be required to determine the precise nature of the DNA sequence alterations that give rise to increased blood pressure.

SOME OTHER "CANDIDATE" GENES FOR HYPERTENSION

In preliminary studies in recombinant inbred strains derived from the SHR and the normotensive Brown-Norway rat, Pravenec et al. have found evidence suggesting the presence of blood pressure regulatory loci in the vicinity of the kallikrein gene family on chromosome 1 (24) and the major histocompatibility complex on chromosome 20 (25). However, it should be recognized that in these studies, the measurements of blood pressure were performed in anesthetized animals and stringent statistical criteria were not employed in the genetic analysis. Accordingly, before firm conclusions about the existence of blood pressure regulatory loci in these regions of chromosomes 1 and 20 can be drawn, the results should be independently confirmed by additional studies in which blood pressure is directly measured in unanesthetized, unrestrained animals.

POSITIONAL MAPPING APPROACH TO EXPERIMENTAL HYPERTENSION: THE EXAMPLE OF ANGIOTENSIN CONVERTING ENZYME

In recombinant inbred strains or in segregating F2 or backcross populations, one can search for chromosome regions that contain genes regulating blood pressure by studying the relationship between blood pressure and the pattern of inheritance of multiple polymorphic markers dispersed throughout the genome (14). By testing multiple markers on every chromosome, it should be possible to identify some markers that are linked to genes regulating blood pressure. Such an approach does not require the investigator to make any initial assumptions as to which genes are most likely to be involved in the pathogenesis of hypertension. With markers that are appropriately spaced along each chromosome, one can assign a blood pressure regulatory locus to a specific segment of chromosome (as defined by the polymorphic markers flanking that segment). As with any linkage analysis, the blood pressure regulatory locus is mapped in terms of centimorgans relative to the flanking markers and its position cannot be determined beyond the megabase level (i.e., beyond the resolution of a few million base pairs of DNA).

In a recent study in an F2 population derived from stroke-prone SHR (SHRSP) and WKY rats, Hilbert et al. (26) and Jacob et al. (27) used a positional mapping approach to search for genes contributing to the pathogenesis of hypertension. In genotyping this population for several polymorphic markers on almost every rat chromosome, these investigators found evidence suggesting the presence of a blood pressure regulatory locus on chromosome 10. Interestingly, the blood pressure locus mapped to a segment of chromosome 10 that was found to contain the gene for angiotensin-converting enzyme (ACE). The relationship between the locus on chromosome 10 and blood pressure appeared stronger in rats ingesting a high-NaCl diet than in those ingesting a standard amount of NaCl. In the F2 population given the high-NaCl diet, this locus appeared to account for approximately 20% of the variation in blood pressure; in the setting of a normal dietary intake of NaCl, the locus appeared to have a relatively small effect on blood pressure. In the same F2 population, these investigators also found evidence suggesting the presence of a blood pressure regulatory locus on chromosome 18 (27).

The success of Hilbert et al. and Jacob et al. in applying the positional mapping approach to search for genes regulating blood pressure is very promising and could represent a watershed event for research on the pathogenesis of experimental hypertension (28). Of course, the results with ACE, like those for other genes that may be linked to the regulation of blood pressure, will need to be independently tested and confirmed by experiments with congenic strains and ultimately with gene targeting approaches. As more polymorphic genetic markers are developed and tested in linkage studies, it is likely that additional chromosome segments will be identified that contain genes regulating blood pressure (Figure 2).
CONSIDERATION OF SEX-LINKED EFFECTS ON BLOOD PRESSURE

In a recent genetic study in congenic strains derived from SHR and WKY rats, Turner et al. found evidence suggesting that the Y chromosome of the SHR might be important in determining the full expression of hypertension (29). In the study in F2 rats derived from SHRSP and WKY, Hilbert et al. also found evidence suggesting a Y chromosome effect on blood pressure (26). Although Y chromosome effects on blood pressure have not been uniformly observed in all genetic studies (30), this possibility clearly merits further investigation. Hilbert et al. have also reported evidence suggesting the presence of a blood pressure regulatory locus on the X chromosome of the rat (26). These investigators found that the SHRSP may have an allele fixed on the X chromosome that contributes to decreased blood pressure (relative to the blood pressure effect of the corresponding allele fixed in their WKY strain). Thus, this X chromosome locus (26) does not appear to be involved in the pathogenesis of hypertension in SHRSP (at least when comparing SHRSP with WKY).

CONSIDERATION OF THE METHODS USED FOR BLOOD PRESSURE PHENOTYPING

In any genetic linkage study, it is essential that the phenotype of each subject be accurately characterized. It has been suggested that some of the problems involved in reproducing linkage studies of mental illness may in part be a consequence of difficulties in accurately determining phenotype (31). Problems in obtaining valid blood pressure measurements may pose the greatest obstacle to successful linkage studies in human and experimental hypertension.

Although many investigators studying hypertension in rats (and mice) employ indirect tail-cuff plethysmography, the validity of this technique is suspect because the method not only requires restraint of the animal, but also the induction of an increase in body temperature (32). Furthermore, by the tail-cuff technique, investigators commonly average only a few pressure measurements obtained on several different occasions. However, blood pressure is a highly labile trait that can fluctuate greatly over time. Thus, it would seem essential to average multiple measurements of blood pressure when attempting to assign a
blood pressure value to a given animal. Of course, the measurement of blood pressure in anesthetized animals should be avoided because of the cardiovascular effects of various anesthetics.

Direct measurements of arterial pressure can be obtained by indwelling catheter techniques. Because of the stress associated with the catheter surgery, measurements of blood pressure should be delayed until several days after the surgical procedure. By computerized monitoring techniques, multiple measurements of blood pressure can then be obtained over prolonged periods. Given the known circadian variation in blood pressure, measurements obtained during the day may need to be examined apart from those obtained at night (33). It is also important to recognize that without scrupulous attention to experimental technique, the long, small-bore catheters commonly used for invasive hemodynamic studies may yield unreliable measurements of systolic and diastolic pressure (because of signal damping effects). The optimal approach may be the use of catheter tip transducers and radiotelemetry devices that allow for continuous, high-fidelity measurements of pressure. At the present time, however, this approach is very expensive and time consuming.

Consideration should also be given to the different implications of measurements of systolic, diastolic, and mean arterial pressure. Increased vascular resistance is the hallmark of essential hypertension and is best assessed by measurements of mean arterial pressure (34). An additional drawback of the tail-cuff method is that it provides estimates of systolic blood pressure only. Systolic blood pressure is determined not only by peripheral vascular resistance, but also by arterial stiffness and left ventricular function (34). Studies that are designed to investigate the increased vascular resistance characteristic of spontaneous hypertension and that depend solely on measurements of tail-cuff blood pressure may be confounded by the effects of other cardiovascular determinants of systolic pressure.

Although it may appear simple to measure blood pressure, genetic studies of hypertension must be carefully evaluated with respect to the methods used to characterize the animals for this complex and labile phenotype. Progress in gene mapping in experimental hypertension will be jeopardized by the failure of investigators to pay serious attention to the techniques used to measure blood pressure. This issue is particularly critical with respect to interpreting linkage studies with negative outcomes. The use of unsophisticated methods to measure blood pressure may yield false-negative results and may lead investigators to incorrectly exclude certain regions of the genome from further consideration in the pathogenesis of hypertension.

**RELEVANCE OF ANIMAL STUDIES TO HUMAN ESSENTIAL HYPERTENSION**

Although it is easier to perform genetic studies of hypertension in rats than in humans, serious questions can be raised about the relevance of such studies to the pathogenesis of essential hypertension. The SHR and Dahl strains were generated by selective inbreeding, a process that could have concentrated rare recessive alleles contributing to increased blood pressure. Obviously, hypertension in humans did not evolve by a similar process. It is unknown whether the genetic basis of hypertension in these rat strains will prove to be similar to that of any form of hypertension in humans.

Given that renin gene polymorphisms may be linked to the regulation of blood pressure in rats, the relationship between renin gene polymorphisms and blood pressure in humans is of particular interest. In a recent study in black Afro-Caribbean subjects, Barley et al. found a possible association between blood pressure and a BglII RFLP in the renin gene (35). Although this finding remains to be confirmed, it raises the possibility that in humans, a blood pressure regulatory locus may exist that is homologous to the one mapped in the vicinity of the renin gene in rats. In contrast to the results in the Afro-Caribbean subjects, no evidence of an association between blood pressure and renin RFLP has been found in white subjects (35,36). In the SHR or Dahl models, the pathogenesis of hypertension may be similar to that in restricted subsets of humans with essential hypertension. Thus, when testing humans for blood pressure regulatory loci identified in animal studies, the etiologic heterogeneity of essential hypertension must be considered. If not, it will be extremely difficult to detect blood pressure regulatory loci that might be relevant only to certain subgroups of patients with essential hypertension.

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