A Paradigm for Identification of Primary Genetic Causes of Hypertension in Rats

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SUMMARY A paradigm is developed for identifying the genes (and the biochemical-physiological traits for which the genes code) that cause differences in blood pressure in inbred strains of rats. A biochemical-physiological trait which meets the following four criteria is one which can reasonably be accepted as causing genetic differences in blood pressure: 1) a difference in a biochemical or physiological trait between two strains must be demonstrated; 2) the trait must be shown to follow Mendelian inheritance; 3) the genes identified in criterion 2 must co-segregate with an increment in blood pressure which is significantly different from zero; and 4) there must be some logical biochemical and/or physiological link between the trait and blood pressure. Traits which do not show discrete phenotypes following Mendelian inheritance may correlate with blood pressure in segregating populations. In this case no rigorous cause and effect genetic argument is possible because such correlations could arise from complex primary genetic causes or as secondary effects of blood pressure on the biochemical-physiological trait. (Hypertension 5 (supp I): 1-198-I-203, 1983)

KEY WORDS • hypertension • genetic • genes

DURING the past two decades several strains of genetically hypertensive rats and mice have been developed. The use of these animal models, especially the spontaneously hypertensive rat (SHR) of Okamoto and Aoki, is widespread in hypertension research. This use has usually not met its full potential because of a failure to include genetic techniques in the analysis of biochemical and physiological traits. The paradigm to be developed here concerns a method for identifying the genetically-controlled biochemical and physiological differences that cause blood pressure differences between genetically hypertensive and normotensive inbred strains.

Polygenic Inheritance of Blood Pressure
Blood pressure is a quantitative trait which is known to be controlled by multiple genetic loci in rats and mice, i.e., blood pressure shows polygenic inheritance. Selective breeding for high or low blood pressure concentrates in the respective strains the genes for high or low blood pressure which happen to be segregating in the base population. Figure 1 shows diagrammatically that selective breeding separates high and low selected lines from the extremes of the base population.

What Constitutes an Ideal Control Strain?
The ideal situation would be to have a hypertensive strain and a control (low-selected) strain which were genetically identical except that at the loci controlling blood pressure the control would carry alleles for low blood pressure in contrast to the hypertensive strain which would carry alleles for high blood pressure. Such an ideal pair of strains does not exist and the selective breeding process for high and low blood pressure from a heterogeneous base population does not result in this ideal situation. Genes at any locus may be selected and fixed (i.e., become homozygous in all individuals of the strain) by chance (genetic drift). This will certainly be the case where inbreeding is practiced for 20 or more generations; essentially all loci will be homozygous and so any genes segregating in the base population may be fixed in one selected strain or the other. The problem is how to differentiate the following: 1) strain differences due to genetic drift; 2) strain differences that are the result selection and which are causally related to blood pressure differences; 3) strain differences that are physiological or pathological responses to the blood pressure differences.

Limitations of Strain Comparisons
The comparison of physiological and biochemical characteristics of a high-blood-pressure strain of rats with a low-blood-pressure control strain is an obvious first step in identifying the factors causing the blood pressure differences. Comparisons of biochemical-physiological traits among high and low blood pressure strains often leads, however, to apparent con-
fusión because of a failure to appreciate the consequences of the polygenic inheritance of blood pressure.

The situation can be illustrated as follows. Suppose that we represent loci controlling blood pressure by the letters A, B, C, X, D, and E. Let a subscript 1 denote alleles (A₁, B₁, etc.), which increase blood pressure by 5 mm Hg (plus alleles) and let a subscript 2 denote alleles (A₂, B₂, etc.), which decrease blood pressure by 5 mm Hg (minus alleles) about a mean of 150 mm Hg. (Obviously it is not necessary that all alleles have the same effect; that is just done for convenience in the example.) Table 1 gives the genotypes for four hypothetical strains which are fully inbred and so they are homozygous at all loci. Suppose also that locus X regulates the activity of an enzyme (enzyme X) whose action actually does increase blood pressure and that X₁ represents the allele for high activity of enzyme X, and X₂ represents the allele for low activity of enzyme X. The blood pressure and enzyme activities generated by the hypothetical genotypes can be calculated from these assumptions, and they are also given in table 1.

By comparing Strains 1 and 2 in table 1, it might be concluded that high enzyme X₁ is associated with hypertension. By comparing Strains 1 and 3, 1 and 4, 2 and 3, or 2 and 4, it might be concluded that enzyme X₁ is not associated with blood pressure differences. By comparing Strains 3 and 4, it might be concluded that low enzyme X₂ is associated with hypertension. The problem is that between strains the effects of many loci are confounded. Some examples of traits for which different conclusions could be reached, depending on the control strains used for comparison in studying hypertensive strains, are: plasma renin; salivary gland renin; urinary kallikrein; reactivity of aortic strips to norepinephrine; catecholamine-synthesizing enzymes in the brain stem; serum dopamine-β-hydroxylase; adrenal steroid 18-hydroxylase activity; 43Ca²⁺ uptake by the aorta; and aortic responses to Cu²⁺.

Biochemical-Genetic Approach

Figure 2 gives the paradigm to be developed for determining the relationship between strain differences in a given biochemical-physiological trait (referred to as trait X) and strain differences in blood pressure. The hypothesis to be tested is: strain differences in trait X cause strain differences in blood pressure. The basic test to be applied is to determine if trait X and an increment in blood pressure are genetically separable. If trait X and blood pressure are genetically separable then trait X cannot be causing blood pressure differences, i.e., the hypothesis can be rejected. If trait X and blood pressure are not separable by genetic manipulation then the hypothesis may be true. The conclusions one can draw from the genetic test depends on whether trait X follows Mendelian inheritance, i.e., shows discrete (discontinuous) phenotypic classes (fig. 2, left) or whether it shows continuous variation (fig. 2, right).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Activity of enzyme X</th>
<th>Blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A₁A₁B₁B₁C₁C₁X₁X₁D₁D₁E₁E₁</td>
<td>High</td>
<td>190</td>
</tr>
<tr>
<td>2</td>
<td>A₂A₂B₂B₂C₂C₂X₂X₂D₂D₂E₂E₂</td>
<td>Low</td>
<td>110</td>
</tr>
<tr>
<td>3</td>
<td>A₁A₁B₁B₁C₁C₁X₁X₁D₁D₁E₂E₂</td>
<td>High</td>
<td>110</td>
</tr>
<tr>
<td>4</td>
<td>A₁A₁B₁B₁C₁C₁X₁X₁D₁D₁E₁E₁</td>
<td>Low</td>
<td>190</td>
</tr>
</tbody>
</table>

See text for assignment of the increments in blood pressure to the various alleles at loci A, B, C, X, D and E, and for assignment of enzyme activity to alleles at locus X.
Mendelian Traits

In the above example of strain comparisons one wants to separate the effect of trait X on blood pressure from the effects of other factors and to establish that trait X causes some increment in blood pressure. To do this it is necessary to meet the four criteria developed below. If these four criteria are met, one moves from the top of figure 2 to the lower left hand corner.

Criterion 1: A difference in a Biochemical or Physiological Trait between Two Strains Must Be Demonstrated

This is merely a statement of the fact that unless a strain difference exists in a biochemical or physiological trait of interest there is no factor to study.

Criterion 2: The Trait under Study Must Be Shown to Follow Mendelian Inheritance

Criterion 2 is not a trivial requirement. Experience shows that most of the work in identifying biochemical-physiological traits causing blood pressure differences will come in identifying a measure which follows Mendelian inheritance. At a minimum this means that discrete, discontinuous phenotypes can be identified in segregating populations. The usual segregating populations studied are F2 and backcross populations. If P1 and P2 are the parental strains, F1 rats are produced from a cross of P1 x P2, backcross to P1 is an F1 x P1 cross, backcross to P2 is an F1 x P2 cross, and F2 rats are produced by an F1 x F1 cross. For example, if inheritance of the trait is codominant then three discrete phenotypes are recognizable, the two parental

HYPOTHESIS: STRAIN DIFFERENCES IN TRAIT X CAUSE STRAIN DIFFERENCES IN BLOOD PRESSURE.

TEST: CO-SEGREGATION ANALYSIS OF TRAIT X AND BLOOD PRESSURE IN F2 AND BACKCROSS POPULATIONS

- Trait X shows discrete phenotypic classes with Mendelian inheritance in segregating populations
  - Phenotypic classes of trait X have different blood pressures
    - Reject the hypothesis
    - Accept the hypothesis
  - Biochemical-physiological links between trait X and blood pressure are evident
    - Test for genetic linkage
      - The association between trait X and blood pressure is retained
        - Accept the hypothesis
      - The association between trait X and blood pressure is lost
        - Reject the hypothesis
  - No biochemical-physiological links between trait X and blood pressure are evident

- Trait X shows a continuous, unimodal distribution in segregating populations
  - Trait X is correlated with blood pressure
    - Reject the hypothesis
  - Trait X is not correlated with blood pressure
    - Reject the hypothesis

FIGURE 2. Flow diagram for applying cosegregation analysis to establish cause-and-effect relationships to genetic hypertension in rats.
types and their hybrid. These segregate 1 parental (P₁) type: 1 hybrid type in a backcross to P₁; 1 parental (P₂) type; 1 hybrid type in a backcross to P₂; and 1 parental (P₃) type: 2 hybrid type: 1 parental (P₃) type in an F₂ population. These ratios are of course altered if one or the other parental alleles exhibits dominance.

Criterion 3: The Genes Identified in Criterion 2 Must Cosegregate with an Increment in Blood Pressure that is Significantly Different from Zero

This test is by far the most powerful. If Criterion 3 is not met, then it eliminates from further consideration traits that follow Mendelian inheritance but do not affect blood pressure. A myriad of such genetic differences will always be found between any two inbred strains because of chance selection and fixation of genes. If Criterion 3 is met, it allows estimation of the size of blood pressure effects of a given locus independently of other loci. Genes at other independently segregating loci influence blood pressure do not interfere with the comparison of the blood pressure effects of the genotypes at a specific locus within a segregating population (usually F₂ or backcross populations). For example, suppose that at a particular genetic locus, call it locus X, which is under investigation for its effects on blood pressure, there are two alleles X₁ and X₂. An animal from a segregating population that is X₁X₁ at locus X may carry genes at other loci for high or low blood pressure; similarly rats from the same segregating population which are X₂X₂ or X₁X₂ at locus X may carry genes at other loci for high or low blood pressure. Since these other genes segregate at random with respect to locus X (assuming that they are not genetically linked to locus X) the net effect on blood pressure of other loci on, for example, a comparison of the blood pressure of X₁X₁ versus X₂X₂ rats within a segregating population will be zero provided a large number of rats are studied.

The problem of cause and effect relationships between a biochemical or physiological trait and blood pressure always lacks definitive resolution in comparing a hypertensive and a control strain. Is the trait under consideration a cause of strain blood-pressure differences, or the result of such differences? If met, Criterion 3 largely solves the issue (with the reservation that closely linked genes are not resolved). A priori the genes controlling the biochemical or physiological trait must be causing any blood pressure differences that co-segregate with them. The blood pressure of a rat cannot determine what genes (and associated phenotypes) it inherited from its parents because the genes were obtained at fertilization before the rats circulatory system was even formed. Note that it is the unidirectional nature of this argument that makes it powerful in establishing cause and effect relationships. The use of this genetic argument depends completely on being able to demonstrate Mendelian segregation of discrete genotypes and associated phenotypes in segregating populations for the biochemical or physiological trait of interest, and associating these discrete phenotypes with blood pressure differences. A mere correlation between a continuously varying (i.e., quantitative) biochemical or physiological trait and blood pressure is insufficient to complete the argument because such correlations could arise from complex primary genetic causes or as secondary consequences of blood pressure; these situations are discussed below.

Criterion 4: There Must Be Some Logical Biochemical and/or Physiological Link between the Trait and Blood Pressure

This criterion is necessary to minimize the possibility that the genetic trait under study actually has no direct biochemical or physiological connection with blood pressure, but co-segregates with an increment in blood pressure because it is closely genetically linked to a locus which really does influence blood pressure.

The problem of linkage is a difficult one. As a reasonable short-cut to a rigorous solution it is logical to accept the alleles involved in determining the phenotypic classes of trait X as the actual cause of the associated blood pressure differences if the association of trait X and blood pressure makes biological sense. On the other hand, if trait X has no clear connection to blood pressure regulation then the association of trait X and blood pressure could still mean that differences in trait X cause differences in blood pressure but the biochemical-physiological mechanism of the association, although real, is just unknown. To differentiate this possibility from a genetic-linkage effect F₂ rats (obtained from crossing two strains polymorphic at locus X) could be bred among themselves at random for several generations. If the association of blood pressure and the genetically-controlled phenotypic classes of trait X is due to genetic linkage, then this association will be lost in subsequent generations due to chromosomal crossing over. If the association is due to the fact that the genes controlling trait X actually affect blood pressure via changes in trait X (or via some other pleiotropic effect of these genes) then the association of blood pressure differences with phenotypic classes of trait X will persist no matter how many generations of random breeding are done. The problem arises as to how many generations of random breeding are necessary to arrive at a decision. This depends on the closeness of the linkage. If the linkage is not close (e.g., 20% chromosomal crossovers) then six to 10 generations will suffice to have a noticeable effect; if the linkage is close (e.g. 1% crossovers) then 60 to 100 generations are required. As a practical matter with rodents, the linkage test is expensive and time-consuming to apply and one could easily end up not being able to discriminate between a biochemical-physiological effect of trait X on blood pressure and a close-linkage effect.

Examples of Genetic Polymorphisms

An example of a genetic polymorphism that meets Criteria 1, 2, and 3 but which does not meet the "bio-
logical sense” test (Criterion 4), was described by Yamori and Okamoto. There are two genetically determined forms of a renal aryl-esterase controlled by a single Mendelian locus in the rat (this locus is known as esterase-4 in the genetic literature). SHR are homozygous for one form and normotensive Wistar are homozygous for the alternate form. The characteristic zymograms follow Mendelian codominance and cosegregate with increments of blood pressure. In F₂ rats the two homozygous types at the esterase-4 locus differed by 12 mm Hg. The function of this aryl-esterase is unknown and so a biochemical-physiological connection to blood pressure is not evident. This may just represent our ignorance about this particular enzyme or it may be that the enzyme serves only as a marker for a linked gene actually influencing blood pressure.

The adrenals of Dahl salt-sensitive (S) rats produce more 18-hydroxy-deoxycorticosterone (180H-DOC) than do adrenals of Dahl salt-resistant (R) rats. The adrenal steroidogenic pathway from deoxycorticosterone to 180H-DOC was shown to be regulated by a single autosomal locus with inheritance by codominance in S and R rats. The locus involved is the structural locus for the adrenal mitochondrial cytochrome P-450, which is an integral part of the 18- and 11β-hydroxylase mechanism. Alleles at the locus involved did segregate with an increment in blood pressure. In F₂ rats, the two homozygous types differed by 16 mm Hg for rats on 8% NaCl diet. Peripheral blood levels of 180H-DOC are two-fold higher in S than R. 180H-DOC is a weak mineralocorticoid which when chronically injected into unilaterally nephrectomized, saline fed rats in physiologic doses increased blood pressure 15-20 mm Hg. Aldosterone can be expected to dominate the mineralocorticoid status of a rat on low and normal salt diets. It has been argued, however, that on high salt diet where aldosterone is markedly suppressed, the mineralocorticoid status of the rat will be determined by steroids from the inner cortical zones (deoxycorticosterone, 180H-DOC, and corticosterone). Thus, in the environment of high salt intake the 180H-DOC production has an influence on mineralocorticoid status, and high 180H-DOC makes the rat more sensitive to salt-induced hypertension. Because the locus controlling 180H-DOC in Dahl rats meets all 4 criteria noted above it is named Hyp-1 for hypertension locus number 1.

The vascular smooth muscle from SHR responds to nonphysiologic cations (Co²⁺, La³⁺, Sr²⁺, Mn²⁺) with a marked contraction whereas various control normotensive stocks do not. In standard genetic crosses between SHR and an inbred strain of Dahl R rats (R/JR) the response of aortic smooth muscle to cobalt (Co²⁺) was shown to be controlled by a single autosomal locus with inheritance by partial dominance. Genes controlling aortic smooth muscle response to cobalt segregated with an increment of blood pressure in F₂ rats. It was estimated that the two homozygous types at the locus involved differed in blood pressure by 15 mm Hg. It is reasonable to speculate that Co²⁺ interacts with the regulatory functions of Ca²⁺ in the vascular smooth muscle and that the use of Co²⁺ unmasks some alteration of Ca²⁺ metabolism in SHR. It will be desirable to prove that this is true, however, by identifying in detail the smooth muscle mechanism involved. The locus controlling vascular smooth muscle response to Co²⁺ was named Hyp-2 for hypertension locus number 2, although it is emphasized that it has not adequately met criterion 4.

Continuous Variation

In the above discussion it was assumed that the biochemical-physiological trait of interest (trait X) showed discrete phenotypic classes which followed Mendelian inheritance. Clearly this will not always be the case. The alternate possibility for a quantitative trait is that it may have a continuous distribution in segregating populations. This possibility is shown on the right side of figure 2, and it would be compatible with polygenic inheritance of trait X (or with mono- or polygenic inheritance with a large environmental variance which obscures the discontinuous phenotypes).

One way of diagraming the relationships between genetic loci and their relationship to their biochemical-physiological links to blood pressure is given in figure 3. In the upper part of figure 3, trait X is controlled by a single locus and the arguments presented above and on the left side of the flow diagram in figure 2 would apply. In the lower part of figure 3, trait X is seen to have inputs from many loci. Mendelian inheritance for trait X would not be found and thus one would be unable to use the arguments developed on the left side of the flow diagram (fig. 2). There would however be a correlation between trait X and blood pressure. The problem is that a correlation between a trait and blood pressure could also arise if differences in the trait were a biochemical or physiological response to differences in blood pressure. Therefore, a mere correlation between a trait X and blood pressure is compatible with the idea that differences in trait X causes differences in

![Diagram](http://hyper.ahajournals.org/DownloadedFrom/1-20x7-to-616x836)

**Figure 3.** Path diagram describing the relationships between genetic loci, their biochemical-physiological traits for which the loci code, and blood pressure.
blood pressure, but it does not constitute a genetic proof of a cause and effect relationship. The best advice in this situation is to look for a new trait related to the area of interest and start again at the top of figure 2 with the new trait, i.e., examine the new trait for Mendelian inheritance. Thus, finding a correlation between trait X and blood pressure although not permitting strong cause and effect arguments to be made, can be very useful in directing further attention to components of trait X which may yield stronger correlation or Mendelian inheritance.

Another possibility is that trait X and blood pressure will not be correlated in segregating populations. This would be evidence for rejecting the hypothesis of a cause and effect relationship between trait X and blood pressure (extreme lower right side of fig. 2).

References

A paradigm for identification of primary genetic causes of hypertension in rats.
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