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

JOHN P. RAPP, D.V.M., PH.D.

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): I-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-
action actually does increase blood pressure and that
X, 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 X is associated with hy-
pertension. By comparing Strains 1 and 3, 1 and 4, 2
and 3, or 2 and 4, it might be concluded that enzyme X
is not associated with blood pressure differences. By
comparing Strains 3 and 4, it might be concluded that
low enzyme X 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;¹⁶ salivary
gland renin;¹⁷ urinary kallikrein;¹⁸ reactivity of aortic
strips to norepinephrine;¹⁹ catecholamine-synthesizing
enzymes in the brain stem;²¹ serum dopamine-ß-hy-
droxylase;²² adrenal steroid 18-hydroxylase activity;²³
⁴³Ca²⁺ uptake by the aorta;²⁴ and aortic responses to
Co²⁺.²⁵

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 differ-
ences, i.e., the hypothesis can be rejected. If trait X
and blood pressure are not separable by genetic manip-
ulation then the hypothesis may be true. The conclu-
sions 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 1. Genotypes for Four Hypothetical Inbred Strains of Rats

<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>A₁B₁C₁X₁D₁E₁E₁</td>
<td>High</td>
<td>190</td>
</tr>
<tr>
<td>2</td>
<td>A₂B₂C₂X₂D₂E₂E₂</td>
<td>Low</td>
<td>110</td>
</tr>
<tr>
<td>3</td>
<td>A₁B₁C₁X₁D₁E₁E₁</td>
<td>High</td>
<td>110</td>
</tr>
<tr>
<td>4</td>
<td>A₂B₂C₂X₂D₂D₂E₂</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

<table>
<thead>
<tr>
<th>Phenotypic classes of trait X have different blood pressures</th>
<th>Phenotypic classes of trait X have the same blood pressures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reject the hypothesis</td>
<td>Trait X is correlated with blood pressure</td>
</tr>
<tr>
<td>Biochemical-physiological links between trait X and blood pressure are evident</td>
<td>Trait X is not correlated with blood pressure</td>
</tr>
<tr>
<td>No biochemical-physiological links between trait X and blood pressure are evident</td>
<td></td>
</tr>
</tbody>
</table>

The association between trait X and blood pressure is retained

<table>
<thead>
<tr>
<th>The association between trait X and blood pressure is lost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accept the hypothesis</td>
</tr>
</tbody>
</table>

FIGURE 2. Flow diagram for applying cosegregation analysis to establish cause-and-effect relationships to genetic hypertension in rats.
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.

**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.27 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 literature28). SHR are homozygous for one form and normotensive Wistar are
homozygous for the alternate form. The characteristic
zymograms follow Mendelian codominance and co-
segregate with increments of blood pressure. In F2 rats
the two homozygous types at the esterase-4 locus dif-
fered by 12 mm Hg.29 The function of this aryl-esterase
is unknown and so a biochemical-physiological con-
nection 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.32 The
adrenal steroidogenic pathway from deoxycorticoster-
one to 180H-DOC was shown to be regulated by a
single autosomal locus with inheritance by codomi-
nance in S and R rats.30 The locus involved is the
structural locus for the adrenal mitochondrial cytoch-
rome P-450, which is an integral part of the 18- and
11β-hydroxylase mechanism.31 Alleles at the locus in-
volved did segregate with an increment in blood pres-
sure. In F2 rats, the two homozygous types differed by
16 mm Hg for rats on 8% NaCl diet.30 Peripheral blood
ing levels of 180H-DOC are two-fold higher in S than R.33
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.32-33 Aldosterone can be ex-
pected to dominate the mineralocorticoid status of a rat
on low and normal salt diets. It has been argued, how-
ever, that on high salt diet where aldosterone is mar-
kedly suppressed, the mineralocorticoid status of the
rat will be determined by steroids from the inner cirtal
zones (deoxycorticosterone, 180H-DOC, and cor-
ticosterone). 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.34

Because the locus controlling 180H-DOC in Dahl rats
meets all 4 criteria noted above it is named Hyp-l 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 normo-
tensive stocks do not.35 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 auto-
somal locus with inheritance by partial dominance.36
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.36 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+ un-
masks 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 hyperten-
sion 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-
genetic inheritance with a large environmental variance
which obscures the discontinuous phenotypes).

One way of diagramming the relationships between
geneic 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 be-
 tween a trait X and blood pressure is compatible with
the idea that differences in trait X causes differences in

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**Figure 3.** Path diagram describing the relationships be-
 tween genetic loci, their biochemical-physiological traits for
which the loci code, and blood pressure.

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**MONOGENIC INHERITANCE FOR TRAIT X AND POLYGENIC INHERITANCE FOR BLOOD PRESSURE.**

**LOCUS A**

**TRAIT A**

**BLOOD PRESSURE**

**MONOGENIC INHERITANCE FOR TRAIT X AND POLYGENIC INHERITANCE FOR BLOOD PRESSURE.**

**LOCUS D**

**TRAIT X**

**BLOOD PRESSURE**

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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 vice in this situation is to look for a new trait related to 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

5. Ben-Ishay D, Saltiel R, Welner A: Separation of two components of trait X which may yield stronger correlation or Mendelian inheritance.

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