Medical Grand Rounds

University of Texas
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GENETICS OF PRIMARY HYPERTENSION

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A routine admission from the renal clinic to the medicine ward.

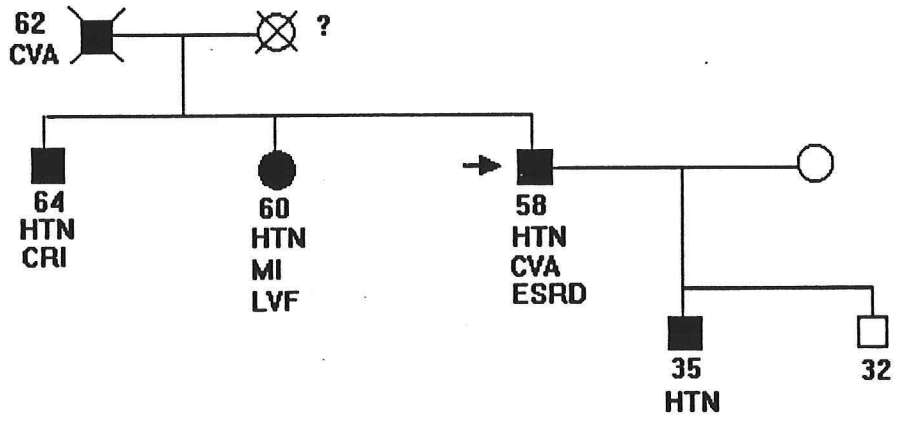
A 57 year old black male who was admitted for
CC: Placement of vascular access for initiation of hemodialysis

HPI: This patient was diagnosed of hypertension in 1978 when he was 42. At the time of presentation, secondary causes of hypertension were excluded and he was placed on β-blockers and diuretics. He was noted to have mild retinopathy and 200mg/day of proteinuria. His plasma creatinine, chest x-ray and electrocardiogram were all reported to be normal.

Over the years, various antihypertensive regimens were tried. Blood pressure control was difficult partly due to multiple reported side effects from medications and to some degree due to non-compliance. In 1989, he suffered a stroke which left him with mild residual left hemiparesis. He had no history of heart failure but has left ventricular hypertrophy on cardiogram and echocardiogram.

In the last seven years, his proteinuria worsened to as high as 1 gm/day and his glomerular filtration rate showed a steady decline. Other causes of renal insufficency and proteinuria were excluded. At the time of admission his serum Cr was 10 mg/dl, and he had bilaterally small kidneys. He was symptomatically uremic with generalized weakness, insomnia, loss of appetite, and pruritis.

The admitting intern documented the patient's family history in detail and accuracy:



In 1761, Morgagni, a physician in Venice, made an observation that could possibly the first account of hereditary hypertension (1). In: *"De sedibus et causes Morboreum per Anatomen Indagatis",* he described,

> *"I noted that the father of one of my patient who died of cerebral hemorrhage also died of apoplexy"*

Although we do not know whether hypertension was the cause of the cerebral events in either one of these patients, this could well be the first recognition of the hereditary nature of hypertension.

Since the time of Morgagni, there is universal accord that hypertension is a significant risk factor for cardiovascular, cerebrovascular, and renal diseases. However, the fundamental etiology of this disease remains largely elusive. Clinical observations such as our case and that of Morgagni strongly suggest that hypertension clusters in families. The scenario of our index case is familiar to all of us. An example is presented of a larger version of the same clinical anecdote taken from a hypertension clinic in France where about 6,600 consecutive charts were reviewed.

	Family Hx of ↑BP			
	Mother or father	Parent + sibling	No family Hx	p
N	1910	854	3830	
(%)	(29)	(13)	(58)	
Age	46	48	51	<0.001
Duration ↑BP	8	10	7	<0.001
Age Dx	38	39	44	<0.001
Sys BP	165	169	168	

In this clinic, roughly two thirds of the patients have family members with documented hypertension. This percentage would likely be higher if blood pressure were systematically taken in all family members rather than assessed by history. Data from all sources collected over the last century from vastly different geographic and socio-ecomnomic areas have uneuqivocally shown that high blood pressure aggregates in families. This clustering is a general phenomonon which occurs throughout the entire blood pressure range. Regardless of whether the proband is hypertensive, normotensive, or hypotensive, the blood pressure of his or her family members will tend to be more similar then that expected by chance alone (2-4).

DEFINITION OF THE HYPERTENSIVE PHENOTYPE

A large amount of effort has been devoted into seeking the dividing line between normotension and hypertension. During the first half of this century, physicians actually believed that blood pressure is a dichotomous trait and an individual is either normotensive or hypertensive. This discreet dividing line is clearly an artifact. Blood pressure is a physiologic variable that spans a continuous range. Clinical hypertension is defined **statistically** as the upper end of a population distribution, or **epidemiologically** as the level of blood pressure that gives rise to cardiovascular and renal complications at a rate that significantly exceeds that of the general population.

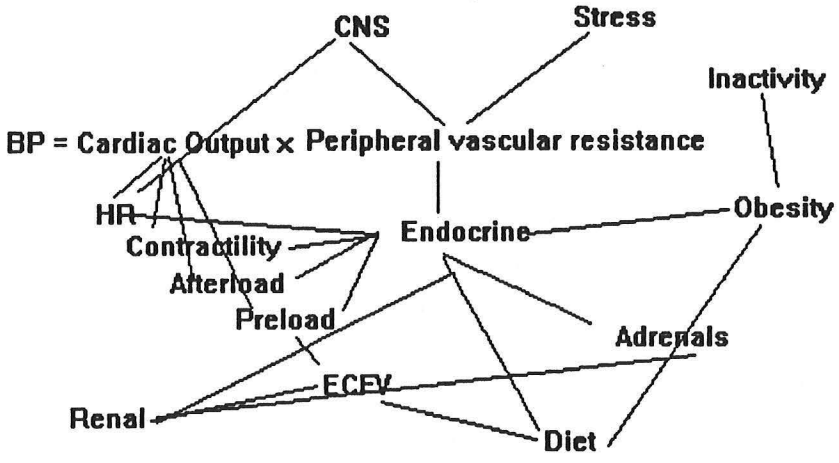
In a simple Mendelian genetic trait, the pattern of inheritance, penetration and expression is relatively staightforward. Both the cellular and clinical phenotypes are often discrete variables. Since hypertension is not an on/off entity, when one considers the genetics of hypertension, one should consider "blood pressure genes" rather than "hypertensive genes". This concept is fitting with the physiologic determinants of blood pressure. For instance, there are probably as many vasodilating substances as vasoconstrictors.

Multiple determinants of BP

Systemic blood pressure is frequently expressed as a simple equation.

Systemic blood pressure = Cardiac output x Systemic vascular resistance

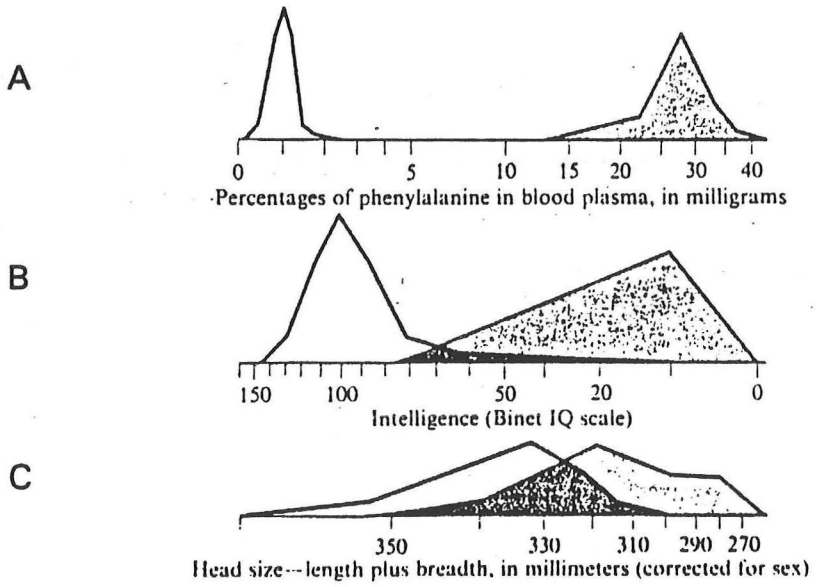
The fact is both cardiac output and systemic vascular resistance are themselves controlled by multiple factors which interact extensively among themselves. All of these factors can have genetic and/or environmental components. A more realistic representation appears more like this.



The net result is that it is very difficult to identify intermediate physiologic parameters that interpolate between the hypertensive phenotype and the underlying pathogenetic mechanisms. In the absence of such physiologic intermediates, the unit of measure of the disease remains blood pressure itself.

Although abnormal physiologic parameters are detected all the time in human hypertension, without well defined pathogenesis, primary alterations cannot be distinguished from secondary alterations. One can attempt to seperate the two by identifying the abnormally before the onset of hypertension. However, initial phases of hypertension in humans are so insidious that they are difficult to detect. Furthermore, functional and structural reactions and adaptation can occur very early rendering seperation of primary and secondary changes extremely difficult.

An excellent example was used by Camuzzi to illustrate the difficulty in studying the hypertensive phenotype (5). Consider a monogenic disease such as phenylketonuria. Here we have a simple mutation that leads to a defect of an enzyme that converts phenylalanine to tyrosine leading to the accuulation of phenylalanine. The figure displays a normal vs. an affected population.



If the proper biochemical intermediate is measured such as plasma phenylalanine, the segregation is clear (A). However, if more distal phenotypic features are assessed such as IQ or head size (B, C), the separation becomes increasingly ambiguous. If we figure head size as a polygenic quantitative trait, the PKU gene will be one of many genes that affect head size. To try to clone the PKU gene with head size as the sole abnormality will be impossible.

IS PRIMARY HYPERTENSION A GENETIC DISEASE

WHY DO WE WANT TO KNOW?

The first and foremost reason is simply to understand the pathogenesis and pathophysiology of hypertension. Although identification of "blood pressure controlling genes" does not guarantee complete elucidation of pathogenesis, it will represent an enormous stride towards understanding primary hypertension.

For diagnosis

The developement of biochemical and genetic markers based on known genetics and pathophysiology will enable screening of early disease in susceptible individuals before the onset of end organ damage. Even with widely advocated blood pressure screening programs, many patients still present with significant end organ disease right at the onset. The identification of the susceptibility genes early in life will prompt more vigilant detection of the phenotype in individuals at risk. Since primary hypertension is a highly heterogenous disease, a classification based on underlying genetic and pathophysiologic defects will be much more valid than any of the existing clinical classifications.

For therapy

Without invoking the prospect of gene therapy, the knowledge of the genetics and pathogenesis of primary hypertension will impact significantly on the design of existing conventional therapies. In addition to initiation of early therapy, the clinician can deliver more specific therapy for their patients. For example, consider two hypothetical patients presenting with systemic hypertension with completely different underlying pathogenetic mechanisms. Patient A has a mutation of a gene whose product regulates the intrarenal renin-angiotensin system leading to slight reductions in glomerular filtration rate and exaggerated tubular reabsorption of Na. In the presence of a liberal salt intake, patient A developes hypertension. Patient B, on the other hand, has a mutation of a gene whose product modulates the signal transduction pathways for neurohormonal vasoconstrictors. This underlying defect, coupled with life style-related stress lead to hypertension in patient B. Theoretically, patients A and B may be distinguished by physiologic measurements early in the course of the illness. However, these primary abnormal physiologic parameters are difficult to ascertain because of the wide range of variation in normals. Second, with established disease, there will remodelling of the vasculature, hypertensive renal damage and cardiac damage rendering these two patient virtually indistinguishable. If the pathogenesis can be established with certainty early by genetic and biochemical markers, patient A would have been treated aggressively with either ACE or AII blockade, natriuretic agents, salt restriction or a combination of the above. Whereas

treatment for patient B would be with specific blockade of the offending vasoconstrictor, a nonspecific counteracting vasodilator, stress reduction, or a combination of the above.

For prognosis

A genetic and pathophysiologically sound classification of primary hypertension will allow the clinician to allot patients in more homogeneous subtypes. The course of the illness, response to therapy, occurance of end organ disease can all be studied and defined epidemiologically. Thus, the identification of certain genetic markers in an individual patient will allow the physician to predict the progression the illness.

GENETICS OF PRIMARY HYPERTENSION: EPIDEMIOLOGIC EVIDENCE

The Mendelian era

The first epidemiologic attempt at studying the inheritance of hypertension was made in the 1920's by Weitz (6). This data can be summarized as follows:

Probands studied		
82 ↑BP	77%	death due to heart disease
267 NBP	30%	death due to heart disease
42 ↑BP	50%	sibling had hypertension, heart disease, or stroke

Weitz concluded that 50% of siblings must have had hypertension. This prompted him to deduce that primary hypertension results from a single, dominantly inherited Mendelian locus. Further follow up of this selected group of patients into the third generation convinced Weitz of the Mendelian pattern of inheritance. Platt in the 1940's basically repeated Weitz's study in an almost identical format and found that 59% of parent of hypertensive probands were hypertensive and in a subgroup of families, the inheritance was strictly autosomal dominant (7). Around the same time Sobye performed a much larger study with similar approaches and found 61% of parent and 23% of siblings of hypertensive probands were themselves believed to hypertensive (8).

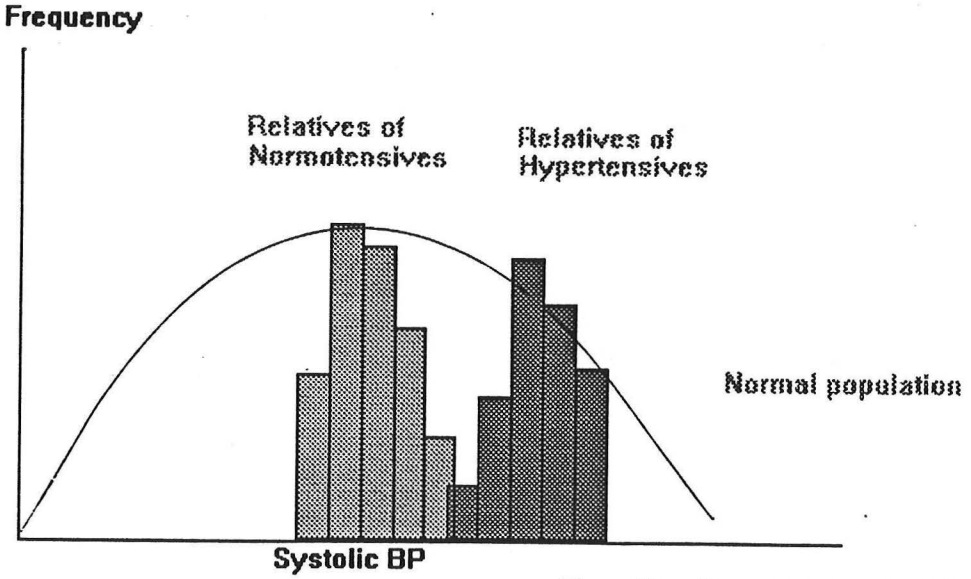
With three independent studies spanning 3 decades arriving at the same conclusion, the case was closed shut and the dogma in the 1940's was that primary hypertension was carried by a single autosomal dominant gene. One problem with these studies is that they were performed on highly selected groups of individuals and in Platt's study, the families that did not exhibit Mendelian patterns through the 3 generations were ignored in the analyses. In addition, the incidence of hypertension in parents and siblings were grossly overestimated because any cardiac or cerebral

event based on hearsay evidence was attributed to hypertension.

Polygenic inheritance

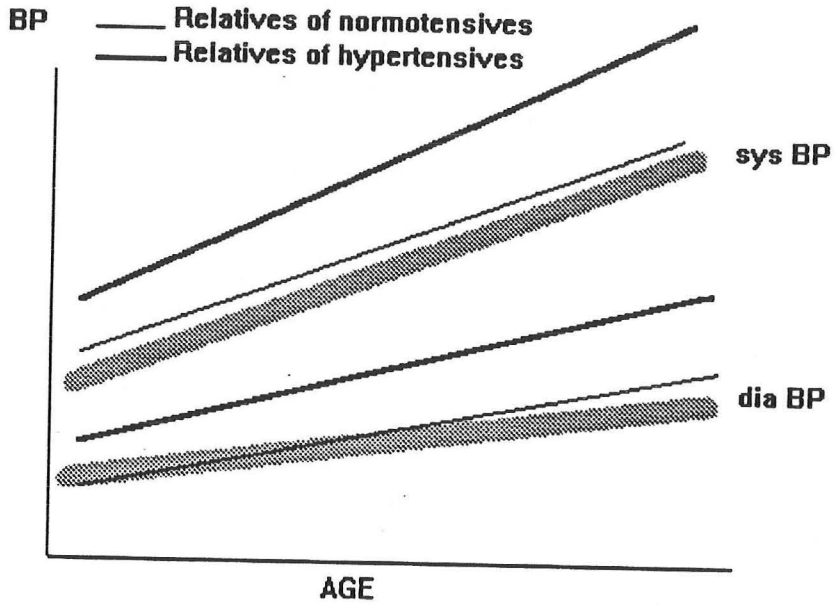
The Mendelian dogma was challenged by a study conducted in St. Mary's Hopsital in London by Hamilton, Pickering, Roberts, and Sowry in the 19050's (9-12). The St. Mary study not only set the methods and standards for studies decades to follow, the basic conclusions drawn from that study still remain intact today. This study was pioneering in a number of ways. The investigators measured blood pressure in 2031 probands that were representative of the population at large. No study prior to this had even considered the possibility of sampling bias. They also were the first to define blood pressure as a continuous variable with hypertension being a tail end of the distribution as opposed to earlier studies that labelled blood pressure as either "high" or "normal". Secondary hypertension was diligently sorted after, and excluded from the study when present. All relatives of the subjects were examined by physicians and blood pressures were measured. Instead of analysing for "raw" blood pressure, they described in detail the age and sex dependence of blood pressure and introduced the concept of age- and sex-adjusted blood pressures. Lastly, they adopted biometrical quantitative genetics to define the relationship between the blood pressure of family relatives in standard statistical terms, thereby allowing hypotheses to be tested.

After defining the blood pressure profile in more than 2000 patients, the investigators selected hypertensives and normotensive by the location of their blood pressure on the distribution curve and studied the blood pressure of their first relatives. These 2 figures illustrate some of their findings.



From Hamilton et al Clin Sci 1954

The data demonstrated that blood pressure is a continuum. Instead of using some assessment of the clinical "hypertensive phenotype" as in the previous studies, these investigators actually measured blood pressure in all the relatives. Clearly, the blood pressure distribution of relatives of hypertensive propositi had blood pressure distributions that were higher than normal.



This correlation exists throughout the entire blood pressure range. The population distribution was used to define age- and sex-specific adjusted scores which is equivalent to todays' z-scores, and the relationship between blood pressure of first degree relatives was evaluated by linear regression of the resulting scores. The following regression coefficients was obtained:

Correlation Coefficient	Within families	Between siblings
Systolic	0.22	0.24
Diastolic	0.18	0.22

These regression coefficient were independent whether the probands were hyper-, normo-, or hypotensive. In general, siblings had higher correlations then parents, and same-sex siblings had higher correlations than unlike-sex siblings. These data clearly contradicted the Mendelian concept and established a new model where polygenic factors accounted for 33% and 22% of the population variability systolic and diastolic pressure respectively.

After the St. Mary's study came other studies that were essentially variations of the original trial. A few representative ones are summarized below.

Relation	Trial Location, year, author	# pats	Regression Coefficient Sys	Dias
Mother-child				
	South Wales UK, 1967 (Miall, 13)	319	0.31	0.30
	Tecumseh USA, 1965 (Johnson, 14)	1496	0.13	0.06
	Framingham USA, 1979 (Havlik, 15)	1495	0.16	0.14
	Polynesian 1979 (Ward, 16)	535	0.14	0.16
	Taiwan 1967 (Tseng, 17)	955	0.11	0.18
Father-child				
	South Wales UK, 1967 (Miall)	273	0.25	0.14
	Tecumseh USA, 1965 (Johnson)	1464	0.16	0.08
	Framingham USA, 1979 (Havlik)	1495	0.14	0.15
	Polynesian 1979 (Ward)	425	0.21	0.17
	Taiwan 1967 (Tseng)	955	0.18	0.23
Siblings				
	South Wales UK, 1967 (Miall)	1088	0.30	0.25
	Tecumseh USA, 1965 (Johnson)	1232	0.17	0.12
	Framingham USA, 1979 (Havlik)	953	0.18	0.18
	Polynesian 1979 (Ward)	1082	0.17	0.13

The gaussian distribution of blood pressure and the mode of vertical transmission uniformly observed in these large trials clearly silenced the Mendelians of primary hypertension. All of these studies concluded that 20-40% of the population's blood pressure distribution was attributable to polygenic factors. The familial aggregation observed traversed diverse ethnic groups living in different ecosystems and it was remarkably stable over time.

The advocates of *Nurture* still claimed that most of these findings merely reflect shared household habits. A few features of these population studies spoke against that fact. In three of the series where correlation was sort for between spouse pairs, the correlation was not significantly different from zero (14, 16, 17). In a few studies where young infants were included, familial aggregation was statistically significant by as early as one month of age (18) or even at birth (19). Therefore, familial resemblance begins virtually at birth or soon after, and is then carried through the rest of the life.

NATURE VS. NUTURE CONTROVERSY, OR IS THERE ONE ?

Today, it is generally agreed that the evidence for familial aggregation of blood is irrefutable (2, 3). However, it is often written that there is still debate as to whether this aggregation merely reflects shared common environments. I believe the controversy does not exist because **both** genetic and environment factors clearly contribute to the familial clustering of blood pressure.

The Nature of the Nurture

There are four factors that have consistently been shown to affect blood pressure.

1. obesity
2. dietary salt intake
3. physical inactivity
4. psychological stress

Since family members tend to share the same lifestyle, all four factors can cluster within families and create a secondary effect on blood pressure aggregation.

Since this discussion is focused on the genetic aspects of hypertension, I will not discuss the original data of environmental impact in detail. In all cases, the impact of the environment on blood pressure is beyond doubt. However, no study has been able to account for all the familial aggregation by environmental factors alone. There are several study designs to demonstrate this effect of nurture. In general, one either maintain a constant genetic pool and vary the environment, or conversely, subject individuals from different genetic pools and subject then to the same environment. These designs are summarized below.

Constant gene pool ***Varied environment***	***Varied gene pool*** ***Constant environment***
Population-based	
Migration studies e.g. comparing first generation Japanese Americans to their cousins who stayed in Japan	Genetically admixed population e.g. studying different ethnic groups within a population
"Westernization" e.g. changes in lifestyle in African or Pacific Island communities with time	Migration studies e.g. Compare first generation immigrants to natives

Family-based

Siblings or twins reared apart
e.g. study whether resemblance is more akin to biologic roots or rearing environment

Adoption studies
e.g. study whether adopted individuals assume the characteristics of the adoptive parents

Animal studies

Rat genetic models of hypertension
e.g. genetically determined hypertensive or normotensive animals reared by mothers from a different strain

Allotting the relative contribution of genes and environment

The adoption study

The adoption study is a quasi-experimental design that examines the impact of a common environment on genetically diverse individuals. One of the most elegantly performed study of this sort was the Montreal French-Canadian adoption study (20-22). This experiment focused on a culturally and ethnically very homogenous population of French-Canadians. The study compared and contrasted families with all adopted chidren, families with mixed adopted and natural children, and families with only natural children. The final cohort consisted of 756 adopted children, 445 natural children, and 1176 parents from a total of 606 families, all within the greater Montreal metropolitan area. A genetic model was applied to the data and the correlations are shown below.

Between	**Correlation coefficient for**	
	Sys	Dias
Parents	0.15	0.18
Mother & natural children	0.27	0.26
Father & natural children	0.24	0.21
Mother & adoptive children	0.08	0.10
Father & adoptive children	0.09	0.13
Natural siblings	0.38	0.53
Adoptive siblings	0.16	0.29

The Montreal French-Canadian adoption study

This study elegantly demonstrated the interaction of nature and nuture. The genetic component was dominating as evident by the high correlation between parent and natural child and between natural siblings vs. the low correlation between parent and adopted child and between adopted siblings. However, the correlation between adoptive siblings were significantly higher than that between parent and adoptive child. In addition, the correlation coefficient betweeen adoptive siblings rose steadily with time such that the coefficients of siblings reared together for 4 or more year were significantly higher than those under 4 years. This implies that the common household environment exerts a significant effect on the blood pressure. The other point to notice is that common household enviromemt has a larger effect on children than on adults because the correlation coefficient between parents remained low even after years together. Overall, the results from this adoptive study indicates that genetic factors accounts for 34% of the population variability in systolic pressure and 30% of the variability in diastolic pressures. The rest of the variation was attributed to environmental factors.

Twin Studies

Another commonly used design to address the impact of shared genes on blood pressure is the twin study. Two representative studies are shown.

Correlation coefficients		Systolic		Diastolic	
		MZ	DZ	MZ	DZ
McIhany (23)	200 children	0.85	0.50	0.80	0.54
Feinleib (24)	514 adults	0.55	0.25	0.58	0.27

Two points are noteworthy. First is the extremely high correlations. Even in the dizygotics where they are only haploid identical, the coeficient far exceeds any determined in natural siblings. This most likely reflects a very strong environmental component shared by twins. Second, the fact that the correlation is less in adults also lend support to the fact that part of the correlation is environmental. The ideal parallel study that will address the role of environment directly will be one examining monozygotic and dizygotic twins reared apart.

Summary

There is little doubt that both genetic and environment factors are important and they interact in the pathogenesis of hypertension. For example, a genetic propensity to salt retention and volume-related hypertension can only be seen with adequate salt in the diet. The relative contributions from genes and environment are variable from population to population. In general, environment tends to have has more impact on diastolic BP and in younger children.

DIFFICULTIES IN STUDYING A POLYGENETIC DISEASE

The two salient features of human primary hypertension are also the two reasons why human hypertension is so difficult to study.

1. The polygenic nature
2. The heavy influence of environmental factors

A genetic disease results from gene mutations that leads to a cascade of events culminating in the morbid clinical phenotype.

gene mutation	→	altered protein function	→	altered cellular function	→	altered organ function	→	clinical phenotype

One usually attack this from the right (the clinical end) because that is the window that the clinician sees. Thus, a logical approach will be:

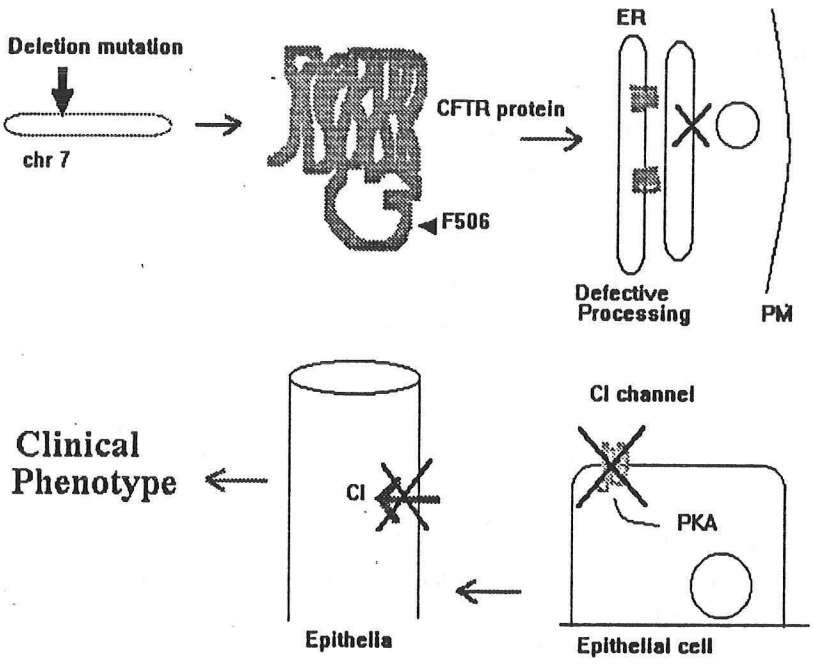
1. Make clinical, laboratory and radiologic observations
2. Define physiological and biochemical abnormalities at whole organism level
3. Define defect at cellular level
4. Identify the protein
5. Clone the gene
6. Identify the mutation
7. Determine if the mutation is causing the disease

Before we apply this reduction approach to human primary hypertension, first consider the complete opposite situation. A single gene disease (autosomal recessive) with minimal contribution by the environment such as cystic fibrosis. In case of cystic fibrosis, the disease in the airways, pancreas and sweat glands was identified as a defect to transport chloride ion (and the accompanying water). At the cellular level, this was identified as an absence of a protein kinase A stimulated Cl conductance in the plasma membrane (25, 26). However, the reduction approach came toa halt at this point and the character of the defective protein escaped all efforts of the epithelial biologists.

The geneticists took a different approach by going directly to the genome using an approach called reverse genetics or positional cloning and by brute force identified the gene that is **linked** to cystic fibrosis. The subsequent characterization of the gene product, the cystic fibrosis transmembrane regulator (CFTR), revealed that it is indeed a protein kinase A-regulated chloride channel.

Now we know a trinucleotide deletion of the CFTR gene (27) leads to deletion

a single amino acid (phenylalanine 506); resulting in defective processing of the protein (28); its absence in the plasma membrane of tracheal, pancreatic, and sweat gland epithelia; absence of Cl transport in these organs; and finally the clinical sequelae that are familiar to the clinician.



In this case, the multidisciplinary approach met each other half way and completed a story of a genetic disease. Cystic fibrosis thus is a good example of everything that human genetic hypertension is not.

1. In cystic fibrosis, the clinical defect was dissected down to at least the cellular level. Human hypertension has not progressed beyond a clinical phenotype. To this day, there are no consistent physiologic intermediates that reliably point towards the pathogenetic origin.

2. A single gene disease such as cystic fibrosis is deemable to successful positional cloning. Positional cloning will be extremely complex or impossible for a polygenic disease such as hypertension.

3. There are few environmental factors that will be significant enough to entirely conceal or mimic the presentation of cystic fibrosis, whereas the hypertensive phenotype can be significantly affected by the environment.

With these sobering facts in sight, do we have any hope of ever sorting out the genetics of primary hypertension? One strategy is to sacrifice likeness to gain simplicity. Go to animal models.

EXPERIMENTAL HYPERTENSION IN RODENTS

Why rodent models?

The sole objective in developing animal models of disease is to provide a theoretical and experimental basis to study the human counterpart. The study of many human diseases are often handicapped by the lack of animal models. In primary hypertension, one cannot invoke this deficiency as an excuse because there are plenty of animal models that offer clear advantages (29).

1. The experimental groups are more homogeneous.
2. The prehypertensive phase can be identified and studied. It is known in an established rat strain whether an animal will develope hypertension or not. This will never be the case with human subjects.
3. Animals can be followed easily throughout their lifespan. Due to the contracted life span of rats compared to human, one is observing a high speed rodent version of the human disease. This is useful particularly in studying the cardiovascular complications of hypertension and following the effects of pharmacologic therapy.
4. Environmental factors can be controlled and there are no compliance problems.
5. One can perform studies to dissect out pathogenetic mechanisms using methods that cannot be performed on human subjects.

How are the models generated?

Genetic hypertension exists naturally in rats and the polygenic nature of rodent hypertension is similar to human. Polygenic hypertension in rats is most likely as complex as human primary hypertension and will be equally difficult to study. However, if an inbred colony is derived by traits selection and specific sibling-mating, the genetic composition of the colony will become very similar. The genes present in the parental hypertensive rats represent a sample of the multiple genes involved in the whole hypertensive population. These genes will be fixed in the offsprings in the inbreedng resulting in a simplification of the polygenic pattern.

As an example, I will described the generation the spontaneous hypertensive rat (SHR) (30). Dr. Okamoto and coworkers at Kyoto University measured blood pressures of several hundred Wistar rats that averaged from 120-140 mm. One male rat with blood pressures ranging from 145-175 mm and one female rat with blood pressures ranging from 130-140 mm were mated and an F1 of 16 males and 20 females were generated. Of the 36 animals, 25 of them had sustained blood pressures of >150 mm. Succesive generations of hypertensive animals were obtained by sibling mating and by the F6 generation, a steady state was reached where rats

spontaneously and uniformly developed blood pressure of 180 mm by 20 weeks of life. There are many different strains of genetically hypertensive rats available and details of their generation is reviewed in ref (31).

Are the models valid?

The single most important factor in this experimental approach is evaluation of the models. The most difficult problem with the evaluation resides in the human condition itself. The major caveat of rodent models of hypertension is that we can never quite verify their relevance against humans since so little is known of the pathogenesis of primary human hypertension.

The traditional method has been to go ahead and breed these animals. When the hypertensive phenotype is obtained, one would measure as many physiologic and biochemical parameters as possible in the animals and compare this whole spectrum to human hypertension.

The reasoning has been: "If what meets the eye is similar, maybe what underlies is also similar". This is not blind faith nor naive wishful thinking. The premise of such extrapolations is based on animal models of secondary hypertension. The best example is the analogy between one form of secondary hypertenion, namely unilateral renal artery stenosis and the Goldblatt two-kidney one-clip model of hypertension (32).

	Animal	Human
Condition	One-clip two-kidney	Unilateral renal artery stenosis
Pèripheral renin activity	increased	increased
Renin activity in		
ischemic side	increased	increased
contralateral side	suppressed	suppressed
Plasma volume	normal	normal
Exchangable Na	normal	normal
Response to AII blockade	fall in BP	fall in BP
Surgical correction or unclipping	fall in BP	fall in BP

As one can see, the two entities are virtually identical except human unilateral renal artery stenosis is usually of insidious onset. In other animal models of secondary hypertension such as mineralocorticoid excess, or catecholamine excess, the physiologic and biochemical characteristics of the animal model also closely parallel those in humans. In these instances, the primary etiology is known in both humans and animal, and the relevant biochemical and physiologic parameters are measured and found to be similar.

The fact that same etiologies give rise to same physiologic parameters in human and animals does not entitle one to reverse the deduction. One must be cautious not to be over confident with such correlative measurements, particularly in genetic models of hypertension. In the absence of precise etiologic factors, any abnormal parameters detected in animal models can be:

1. Directly causing hypertension
2. Not directly causative but a reflection of pleiotropism of a gene causing hypertension
3. An intermediate between the underlying causative mechanism and the phenotype
4. Genetically linked to the causative gene hypertension but plays no pathogenetic role
5. A result of hypertension.

Shown on the next page is an **incomplete** summmary of the abnormalities detected in one of the rodent models, namely the spontaneously hypertensive rat (SHR).

The exhaustive detection of abnormalities in a rodent model is clearly NOT the right experimental approach to understanding hypertension. This list is as complex and confusing as the list of abnormalities in human hypertension. If the purpose for using an experimental model is to simplify, this approach will be an outright defeat.

So why should one replace a complex human disease with an equally complex and perplexing rodents rodent model whose relevance to human disease cannot be determined. In other words, is rodent genetic hypertension worthless? The answer is no.

A partial list of abnormalities detected in the SHR

Peripheral vasculature
- ↑ neurogenic vasoconstriction
- ↑ resting tone
- ↑ vasopressor response to AII, NE, AVP
- ↓ vascular lumen in resistance vessels
- ↑ vascular smooth muscle proliferation
- ↑ cell pH VSMC
- ↑ Na/H exchange in VSMC

Membrane transport defects defined mainly in erythrocytes & leukocytes
- ↑ cell Na (some);
- no change cell Na (some)
- ↑ cell Na after salt loading
- ↑ RBC membrane Na permeability
- ↓ RBC Na-K cotransport with ↓K_{Na} (some)
- ↑ RBC Na-K cotransport (some)
- ↑ RBC and WBC Na-Li exchange
- ↑ WBC Na/H exchange
- ↓ RBC NaKATPase; presence of circulating inhibitor
- no change NaK ATPase
- ↑ RBC cell Ca & ↓ Ca binding to membranes
- ↓ calmodulin-stimuated Ca transport

Neurogenic
- enlargement of neurons of the subfornical organ and the organ vasculorum lamina terminalis
- ↑ responsiveness and receptor binding to AII
- ↑ brain tyrosine hydroxylase
- ↑ brainphentolamine-N-methytransferase
- ↓ brain NE turnover in young but ↑ in old
- ↑ muscle NE turnover in young but ↓ in old

Endocrine
- Hyperplasia and hypertrophy of adenohypophysis
- Hyperplasia and hypertropy of adrenal zona glomerulosa and fasiculata
- ↑ circulating aldosterone and corticosterone (some)
- Hyperresponsiveness to ACTH and KCl
- no change in circulating aldosterone and corticosterone
- ↑ circulating AVP (some)
- ↓ circulating and brain AVP (some)
- ↓ ANP in atria
- ↓ ciculating ANP but ↑ ANP response to Na load
- ↑ thyroid hormones (some)
- no change in thyroid hormones but amelioration of ↑BP by thyroidectomy (some)

Renal
- SHR donors renders normal recipients hypertensive
- ↓ GFR and SNGFR (↓RPF); ↑TG feedback in young
- Progresibe increase in filtration barrier with age
- ↓ urinary kallikrein
- ↓ FE^{Li} (some); no change (some)
- ↑ proximal NaCl absorption and ↓ distal delivery

How does one use these rodent models?

In order to effectively use such models to generate knowldege that is relevant to human primary hypertension, one needs to go through three experimental stages.

1. Establish resemblance

First, one needs to evaluate if a particular animal model resembles at least a subgroup of patients with essential hypertension. There are actually a great deal of similarity between rodent models humans. Blood pressure rises with age in both conditions resulting in cardiovascular complications such as left ventricular hypertrophy, congestive heart failure, strokes, and nephroschlerosis.

One important difference exists. Because hypertensive rodents are generated by inbreeding, the number of genetic determinants of the hypertensive phenotype is much more restricted compared to humans. This feature is an advantage because it allows the investigator to focus in on certain aspects of the pathophysiology free from excessive number of confounding factors.

Myriads of abnormal physiologic parameters are present in all strains of genetically hypertensive rats. In each strain, there are specific predominant features that often resembles certain clinical subgroups of patients.

Strain	**Predominant abnormalities**
Spontaneous Hypertensive Rat *[SHR]*	Increased PVR. Early: central neurogenic vasocontriction Late: mainly structural vascular changes with accelerated smooth muscle growth.
Stroke-prone SHR *[SP-SHR]*	Greatly increased sympathetic tone Accelerated hypertension with high incidence of CVA's.
New Zealand Genetically Hypertensive *[GH]*	Cardiac hypertrophy Increased PVR, abnormal Ca and prostaglandin metabolism
Sabra Hypertensive *[SBH]*	Na and H_2O retention

Milan Hypertensive *[MH]*	Small kidneys, renal Na retention Adrenal hyperplasia and high aldosterone
Lyon Hypertensive *[Lyon]*	Genetic defect in CNS function Genetic dyslipoproteinemia
Dahl salt-sensitive *[DS]* salt-resistant *[DR]*	NaCl-induced ↑BP Abnormal pressure natriuresis Defect in baroreceptor reflex Increased sympathetic tone
Fawn-hooded *[FH]*	Malignant nephroschlerosis Chronic hypertensive nephroschlerosis

2. Study the model until a satisfactory conclusion can be drawn

After establishing that the animal model is relevant to at least a subgroup of patients with primary hypertension, the next step is to use whatever physiologic, biochemical, pharmacologic, genetic, and molecular methods at the disposal of the investigator to try to dissect out the underlying pathogenetic mechanism in the animal model.

3. Return to human hypertension

This may be the most enlightening or disappointing of the 3 steps: to apply the knowledge to the subgroup of patients to test if the same pathogenetic mechanisms apply.

An excellent example of the application of rodent models to human hypertension following this three-step approach is in studying the role of vascular smooth muscle in the pathogenesis of hypertension.
Folkow and others have pointed out the two key changes in blood vessels in human essential hypertension; increased mass of resistance vessels, and increased reactivity to vasoactive substances (32). The work with rodent models has significantly advanced our understanding of the vasculature in hypertension. For an excellent review, see ref 33.

GENETIC APPROACHES TO HYPERTENSION

Primary hypertension in humans have defied attempts to identify the pathogenetically important genes. The combination of classical genetics and molecular biology has provided powerful methods to study the genetics of primary hypertension. Each of thèse approaches will be.

1. Candidate gene approach
2. Genomic screening and poistional cloning
3. Trangenic manipulation

Candidate gene approach

This approach has been used in both experimental animals and humans. The principle rests on stepwise attempts to satisfy the following criteria:

1. **The conception that the gene product provokes hypertension has to have a physiologic basis.** This means that there should be some understanding of the physiology of the candidate gene product in blood pressure regulation.

2. **There is a phenotypic difference between hypertensives and normotensives.** Clinically, the measurable biochemical trait linked to the gene product should be different between hypertensives and controls.

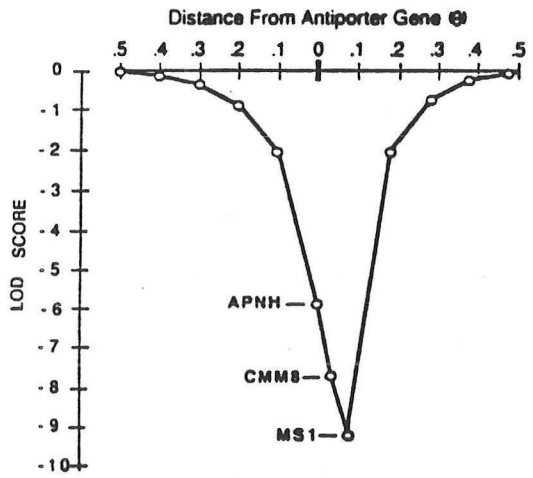
3. If a candidate gene is suspected in the pathogenesis of hypertension, its structure or sequence can be then be examined for possible differences within a population. This allelic variance is called polymorphism. **The polymorphism of the gene should cosegregate with variations of blood pressure.** A detailed study of the polymorphism is usually performed in a population of normo- and hypertensives and a difference in the polymorphisms may be established. In addition, well-defined families with hereditary hypertension will also be studied to search for segregation of the alleles**.

** A cosegregation analysis is an attempt to establish the physical distance between the candidate gene and the disease causing gene by probability. If they are one and the same gene, this distance will be zero. If the two genes are situated on two different chromosomes, the candidate gene will be associated with the morbid phenotype in a random fashion. The candidate gene is thus said to be in "linkage equilibrium" with the disease gene. In contrast, the two genes are situated on the same chromosome, it will be found disproportionately often in association with the morbid phenotype. Due to meiotic recombination events, this assocaition will not be perfect. The frequency of recombination between two loci is a function of the physical distance on the chromosome. A 1% recombination rate represents one centiMorgan units (cM) which is approximately 10^6 base pairs. For a recombination fraction of θ, the ratio of the odds of obtaining a particular set of linkage data over the odds of obtaining the same data purely by chance can be deduced. The logarithm of this likelihood ratio is termed the Lod score. A Lod score greater than or equal to 3 indicates a 1:1000 chance that the linkage had occurred by chance. A lod score of -2 or less usually indicates absence of linkage.

The candidate gene approach cannot establish a causal link between hypertension and the candidate gene. If this is a cause-and-effect relationship, further analysis of particular cosegregating allelic patterns should reveal a mutation which affects structure and function of the gene product in a way that contributes to the morbid phenotype. This is an important confirmation particularly when this approach is applied to inbred hypertensive animals. Loci that are totally unrelated to pathogenesis of hypertension can be fixed by chance due to the inbreeding, a phenomenon known as genetic drift.

The Na/H exchanger (NHE-1) was ruled out as a candidate in human hypertension

One example of the candidate gene approach used in humans was the Na/H exchanger. One of best known "intermediate phenotype" in human hypertension is the elevated Na/Li and Na/H countertransport (exchange) in RBCs (35) and platelets (36). Elevated Na//Li exchange is absent in secondary hypertension suggesting that it is not a result of elevated blood pressure (35). This trait is also found in normotensive offsprings of hypertensive parents (37) and it confers an increased risk of developing hypertension (38). Pedigree analysis has shown that in certain subgroups of patients, this major locus displays incomplete recessive inheritance (38). It has been suggested Na/Li exchange occurs via the Na/H exchanger with Li substituting for H. Although the abnormal Na/Li exchange in RBC is by itself is non-pathogenic, it serves as a flag of an more generalized Na/H exchange activity defect due to a mutation in the Na/H exchanger gene (39). Such abnormalities can account for increased Na absorption in the kidney and/or increased contractility in vascular smooth muscle cells. This makes the Na/H exchanger a good candidate gene to be tested. When the gene of the first isoform of the Na/H exchanger NHE-1 was cloned (40-42), it was immediately tested in the Utah pedigrees using Na/Li exchange as the intermediate phenotype (43).



The results were negative. Using polymorphism of a *TaqI* restriction fragment of NHE-1 itself along with 2 neighboring highly polymorphic markers CMM8 and MS1, the LOD score for NHE-1 (labelled in figure as APNH) was -5.91. A million to one chance that the NHE-1 gene is linked to abnormal Na/Li exchange. When BP was tested as a quantitative phenotype, simialr results were obtained. This was further supported by a hypertensive sib-pair analysis.

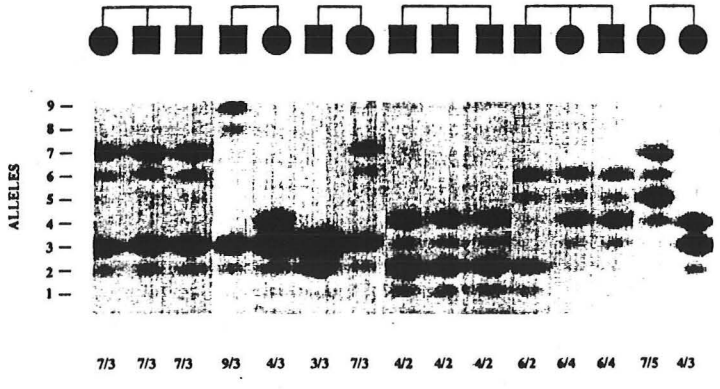
Although a positive result might have been more exciting, ruling out candidate genes are as important as ruling in candidates genes in a polygenic disorder. A number of candidate genes have fulfilled criteria 1 and 2 but failed criteria 3 when linkage was examined. This includes ACE and renin. The possibility exists still of other genetic and environmental factors that can modulate Na/Li exchange by a sufficient degree that it became impossible to document linkage.

The angiotensinogen gene was identified as a candidate in human hypertension

The next example illustrates a positive candidate gene study as well as a different type of linkage analysis. The candidate gene of interest is angiotensinogen. This was based on the fact that angiotensin levels are elevated in hypertensives probands and their relatives compared to normals in some poulation (44, 45). Within some hypertensive populations, angiotensinogen levels directly correlates with BP levels (46). Infusion of angiotensinogen does ellicit a hypertensive response and infusion of anti-angiotensinogen antibodies causes hypotension (47). Therefore the first two criteria for candidate gene is fulfulled.

Instead of ultilizing extended pedigrees, this study used the sibling pair analysis that was first used in diabetes mellitus in the 1970's (48). This approach presumes no a priori formulation of a genetic model of inheritance. The principle of the method relies on the fact that even if a phenotype is affected by multiple genes, any one candidate gene will always follow a simple Mendelian inheritance pattern. One would predict the number of alleles shared by the affected siblings based on the frequency of the alleles in the population under the null hypothesis of independent assortment of trait and marker. Then one simply compares the observed allelic sharing to the predicted value and draw conclusions based on statistical evaluation of the difference. Therefore, all that is required for this method is the test population and the cloned candidate gene.

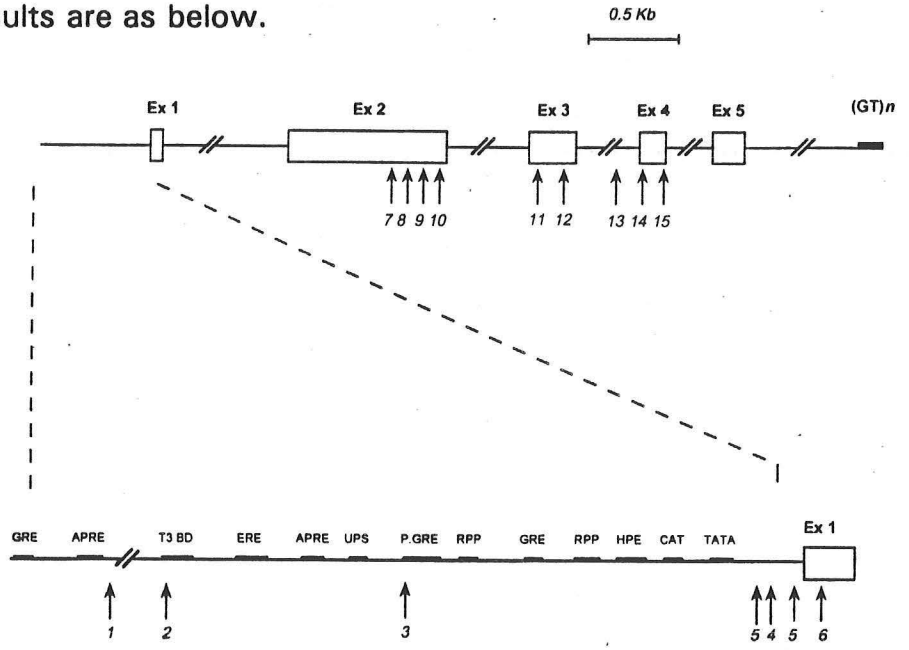
A total of 244 sib pairs from Utah and 135 sib pairs from Paris were evaluated (49). Genotyping with a dinucleotide (GT) tandem repeat at the angiotenogen locus showed 15 different alleles. Seven of these are shown below as variant PCR fragments due to different number of GT repeats from the same set of primers.



Sib pair analysis showed the following results.

Test population	Alleles shared		Excess	p
	Observed	Expected		
Utah	312	303.6	3.8%	0.11
Paris	175	162.6	7.7%	<0.05
TOTAL	487	466.2	5.1%	0.02

The allelic sharing in the Utah population by itself did not reach significance, but the Paris population alone or the combined Utah and Paris population did show significant excess allelic sharing. This may be due to the fact that the Utah patients were recruited from the general population while the Paris patients were from a tertiary referral center. When a subgroup of severe hypertensives (2 drugs or more) from Utah was studied, the excess allelic sharing was 18% with $p<0.01$. These investigators then went ahead and defined each of the 15 alleles on the angiotensinogen gene and the results are as below.



Some of these are in noncoding and possibly regulatory regions (1-5, 13) and some are in coding regions (7-12, 14-15). Two alleles T174M and M235T that led to single amino acid changes were particularly prevalent in the whole population and their frequency is significantly increased in the hypertesnive population. These two variant exhibited complete linkage dysequilibrium with respect to each other.

	Allelic frequency (q)	
	T174M	M235T
Control	0.09	0.36
All index cases	0.14 ($p<0.05$)	0.47 ($p<0.001$)
Severe index cases	0.17 ($p<0.01$)	0.51 ($p<0.001$)

Interestingly, when plasma angiotensinogen levels were studied as a function of the M235T allele by analysis of variance, the M235T allele appeared to exert a codominant effect on elevated plasma angiotensinogen levels.

Influence of the M235T Variant on Plasma Angiotensinogen Concentrations

M235T	AA	Aa	aa	Significance (F, *p*)
Salt Lake City	1422 ± 247 (67)	1479 ± 311 (109)	1641 ± 407 (33)[a,b]	5.92, p<0.005[c]
Males	1376 ± 247 (42)	1404 ± 265 (59)	1499 ± 207 (18)	1.53, ns
Females	1500 ± 232 (25)	1566 ± 340 (50)	1811 ± 519 (15)[a,b]	3.91, p<0.02
Paris	1085 ± 210 (32)	1318 ± 383 (55)[d]	1514 ± 511 (29)[a,b]	7.90, p<0.001[c]
Males	1086 ± 244 (17)	1311 ± 290 (26)[d]	1377 ± 606 (10)[a]	2.82, p = 0.07
Females	1084 ± 173 (15)	1324 ± 456 (29)[d]	1586 ± 455 (19)[a,b]	6.44, p<0.01
Total	1313 ± 283 (99)	1425 ± 344 (164)	1582 ± 459 (62)[a,b]	14.9, p<0.0001[e]
Males	1293 ± 277 (59)	1375 ± 274 (85)	1456 ± 391 (28)[a]	3.1, p<0.05
Females	1344 ± 292 (40)	1477 ± 401 (79)	1685 ± 490 (34)[a,b]	6.82, p<0.001

Plasma angiotensinogen concentrations are expressed as mean ± the standard deviation (nanograms per milliliter). A, allele M235; a, allele M235T. The statistical significance tested by one-way analysis of variance is unmarked.

[a] p<0.05 between homozygotes M235T and homozygotes M235.

[b] p<0.05 between homozygotes M235T and heterozygotes.

[c] The statistical significance tested by two-way analysis of variance with gender as a fixed effect.

[d] p<0.05 between heterozygotes and homozygotes M235.

[e] The statistical significance tested by three-way analysis of variance with gender and population as fixed effects.

This is the first study in humans that linked a particular gene to the clinical phenotype. The significance of the findings are still speculative at this point. The same investigators also found an association of this variant with patients with pre-eclampsia. A hypertensive disease that may be estrogen related but not genetic in origin (50).

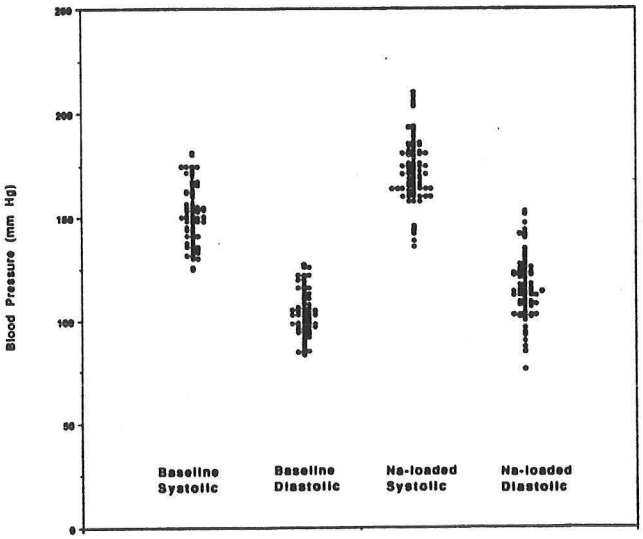
Genomic screening and positional cloning

This is based on the same principles of linkage as the candidate gene approach, except there are no specific candidate genes. Instead, a multitude of markers scattered throughout the genome is used to examine if any one of them is linked to the morbid phenotype. One marker (or markers) that maps closest to the disease locus (or loci) is found, then physical mapping techniques such as chromosome jumping and walking is used to maximally narrow down the region of interest. As mentioned above a number of monogenic diseases have been defined this way. This includes Duchene muscular dystrophy, cystic fibrosis, type I neurofromatosis and autosomal dominant polycystic kidney disease. One has to keep in mind that the distribution of the markers may not cover the entire genome, large "blind-spots" between markers may be missed.

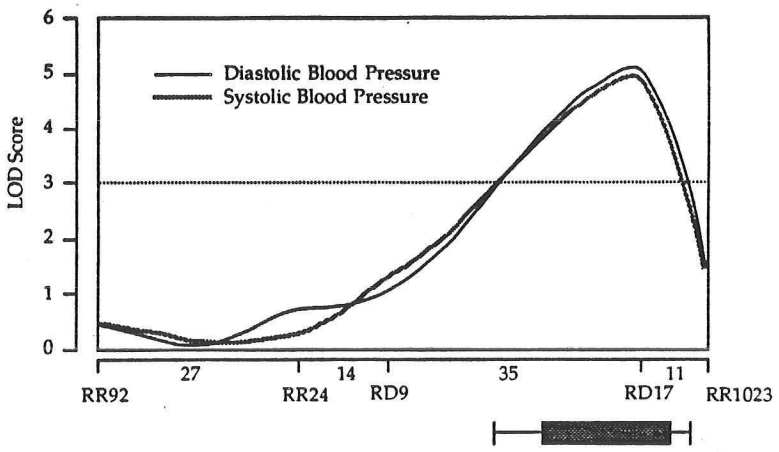
This approach although daunting, is feasible with monogenic diseases. In human hypertension, it becomes virtually impossible. One possible scenario where there may be a chance of success is in animal models of polygenic hypertension where the genetic component has been simplified. By reducing the genetic heterogeneity and by using a sufficiently large number of animals, one can achieve the statistical power to detect genes of a quantitative trait.

The "shotgun" approach identified linkage of ACE with hypertension in SHR-SP

This was done with the stroke-prone spontaneously hypertensive rats (SHR-SP) (51, 52). SHR-SP was crossed with the WKY normotensive controls and an F2 progeny of 114 rats were produced. These rats showed a scatter of blood pressure measurements and displayed salt sensitivity that is characteristic for SHR-SP.



Scatterplot of F2 BP



LOD score of linkage between BP markers

Since the rat genomic map was rudimentary compared to the mouse, new markers were developed which consisted of either minisatellites of repetitive 10-100 bp elements or microsatellites of di-, tri-, or tetranucleotide repeats. The number of repeats of these satellites are highly polymorphic and can be identified easily by PCR once the flanking sequences were cloned. Using a panel of 240 mini- and microsatellites, the F2 cohort showed significant linkage of several markers to hypertension. The highest LOD was the marker RD17(GH) which spanned 16 cM that roughly covers the 1.0 LOD support interval (or a 10-fold drop in likelihood ratio). When the ACE locus RD31 was genetically mapped in the SHR-SP x WKY intercross, RD31 (ACE) was tightly linked to RD17(GH) with 0% (0/224) recombination.

This locus seemed to elevated Na-loaded BP much more than baseline BP. The authors have postulated a failure of Na suppression of the renin-angiotensin system as a possible explanation. In another study, when Dahl salt-sensitive rat were crossed with Milan normotensive, ACE was found to be linked to hypertension in in F2 rats using a candidate gene approach (53). Classical physiology has taught that renin, not ACE, is the rate-limiting step in AII generation. While this is true for circulating renin, this may not be true for the autocrine and paracrine renin angiotensin systems in the heart, vasculature and renal epithelia. ACE activity is elevated in the aorta of SHRs (54). The pathophysiology of the role of ACE in SHR-SP and Dahl rats remains to be defined.

A recent study using two human alleles of ACE and the tightly linked (LOD 11) highly polymorphic human growth hormone gene as a surrogate, found no evidence to support linkage between the ACE locus and the test population in Utah (55). Given the sib-pair approach, the high number of sibs, and an extremely polymorphic surrogate marker, the power to detect linkage was very strong. Yet the authors were cautious to reconcile their negative data with the rat data by stating: 1. BP was studied as a dichotomous rather than a quantitative trait, 2. only baseline BP and not salt-loaded BP was measured, 3. the test population was restricted in ethnic diversity.

Transgenic manipulation

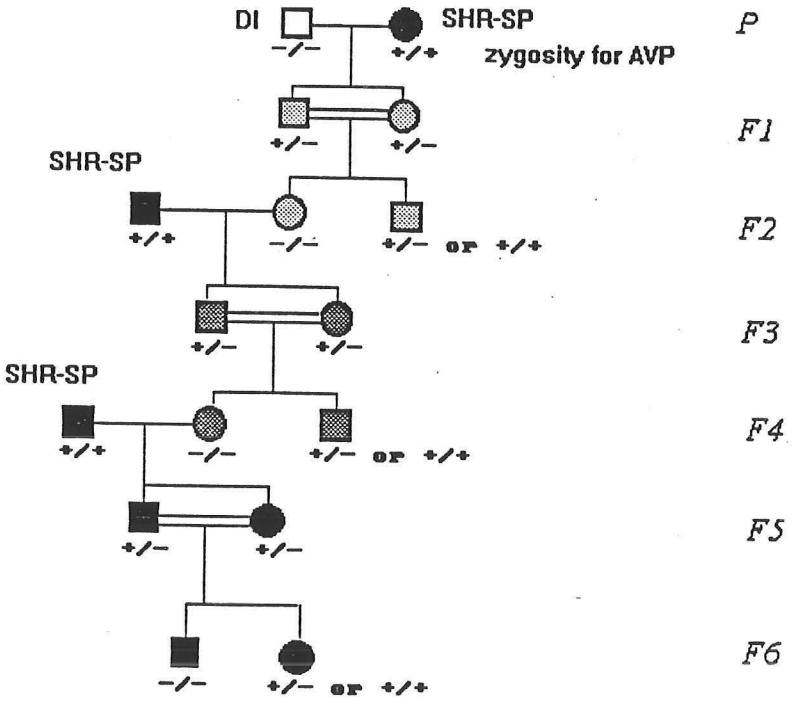
When loci are identified by linkage, a whole series of new experiments will have to be performed. The ultimate specific test of a candidate gene is by expressing it in transgenic animals and examining the phenotype. This will be discussed under its own section.

TRANSGENIC ANIMALS: GETTING TO SPECIFIC GENES

Getting down to specific genes: the SPSHR-DI experiment

The first example that invesigators attempted to manipulate a single gene in rodent hereditary hypertension was the work of Ganten and colleagues back in 1978 (56). The stroke-prone spontaneously hypertensive rat (SHR-SP) exhibits severe hypertension with adult BP's of over 230 (57) and were so named because over 90% of them end up suffering from hypertensive strokes. Because AVP levels were suppressed in the circulation as well as in the brain of these animals, Ganten formulated the hypothesis that AVP is not essential for the development of hypertension and went ahead to prove this point with a simple but ellgant classical genetics experiment.

This was a gene knock-out experiment performed in 1978 using what nature provided. The Brattleboro rat is a strain of rats with hereditary hypothalamic diabetes insipidus (DI). This defect is due to a single base deletion in the second exon of the AVP gene that induces a frame-shift giving rise to a nonsense translational product and an inactive precursor of AVP.



SHR-SP's were bred with Brattleboro rats to introduce the null AVP genotype into the hypertensive rats. In doing so, the hypertensive gene pool was diluted (F1). To enrich the SHR genes once again, the F2 was back crossed with the parental strain of SHR-SP. After five to six, one could attain "hypertensive gene densities" comparable to the pure SHR-SP. By mating these hypertensive rats heterozygous for DI, a double AVP knock-out can be acheived in SHR-SP. Blood pressure in SHRSP/DI developed essentially the same way as in SHRSP with respect to absolute levels, time course, and complication rates.

The mortality of the SHRSP/DI's were very high mainly because in addition to getting strokes, these animal frequently die of dehydration.

The insertion of the defective AVP gene into SHR clearly demonstrate that AVP

is not essential for the development and maintenance of hypertension in SHR-SP. Although this was a negative study, this represented the first example of manipulating a specific gene in any model of hypertension. However, to wait for the rare serendipitous event of a spontaneous mutation is too cumbersome and uncertain.

The use of transgenic animals to examine the role of specific genes in hereditery hypertension

The renin-angiotensin system will be used as a paradigm for transgenic animals because it is the most extensively studied system in terms of physiology as well as molecular genetics (58, 59).

Brief review of the physiology:

Angiotensinogen---------→ AI----------→ AII----------→ AII receptor
renin ACE

Angiotensinogen is primarily synthesized in the liver as a high molecular weight precursor of angiotensin under the stimulation of estrogens, glucocorticoids, thyroid hormone, and angiotensin II. Renin cleaves the inactive decapeptide prohormone AI from the amino terminus of angiotensinogen. AI is then converted through angiotensin converting enzyme, a dipeptidyl carboxypeptidase, into the active octapeptide AII which acts on receptors distributed widely in in multiple organs including the brain, the arteries, the adrenal gland, the kidneys and the heart.

Summary of genetic data of RAS in rats and humans.

ANGIOTENSINOGEN

1. A molecular variant of angiotensinogen is linked to patients with human hypertension.
2. The same variant is linked to pre-eclampsia in humans.

RENIN

1. A polymorphism cosegregates with blood pressure in Dahl rats in a "dose-dependent" fashion
2. Gene structural alteration in SHR-SP and SHR compared to WKY
3. Lack of linkage to hypertension by RFLP in humans

ACE

1. Hypertensive locus mapped in SHR-SP is tightly linked to ACE.
2. Lack of linkage by sib-pair analysis in humans

Why rats instead of mice?

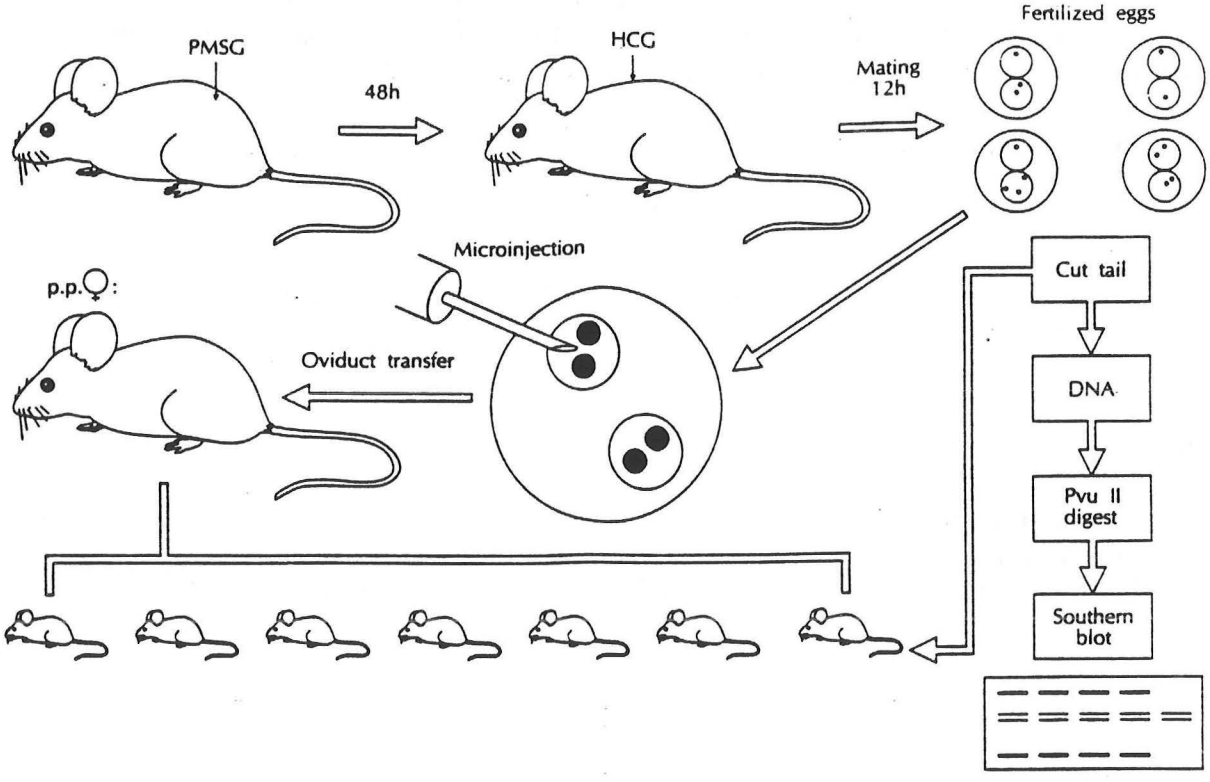
Mice has traditionally been used for expressing transgenes for the following reasons:

1. mouse genetics well known
2. injection of genes into mouse oocytes is technically well established
3. possible to delete genes by homologous recombination in mouse embryonic stem cells

However, mice are less than ideal for the following reasons:

1. primary hypertension is virtually non-existent in mice.
2. although blood pressure measurements can be done, performance of sophisticated cardiovascular tests in mice is technically infeasible.

The rat has been used more often than any other species in pathophysiologic and therapeutic research in hypertension. Rats are known in nature to develope primary hypertension with end organ damage. Transgenic rats can be studied with established methods in the physiology, hemodynamics, electrophysiology, endocrinology, and pharmacology of hypertension.



Generation of transgenic rats

Approximately 30 day old rats are superovulated by pregnant mare's serum gonadotrophin and human chorionic gonadotropin and mated with male rats. The rats are then sacrificed. The swollen ampulla of the oviduct is opened and the fertilized oocytes are isolated and prepared for injection. After formation of pronucleii, the oocytes are injected with DNA and are re-implanted into the oviduct of pseudopregnant 16 week old female Sprague-Dawley rats which have been mated with vasectomized males before the oocyte transfer. The presence of the transgene in the ofspring of these animals is confirmed by obtaining tissue from the animal and using Southern blots or polymerase chain reaction.

Transgenic expression of angiotensinogen

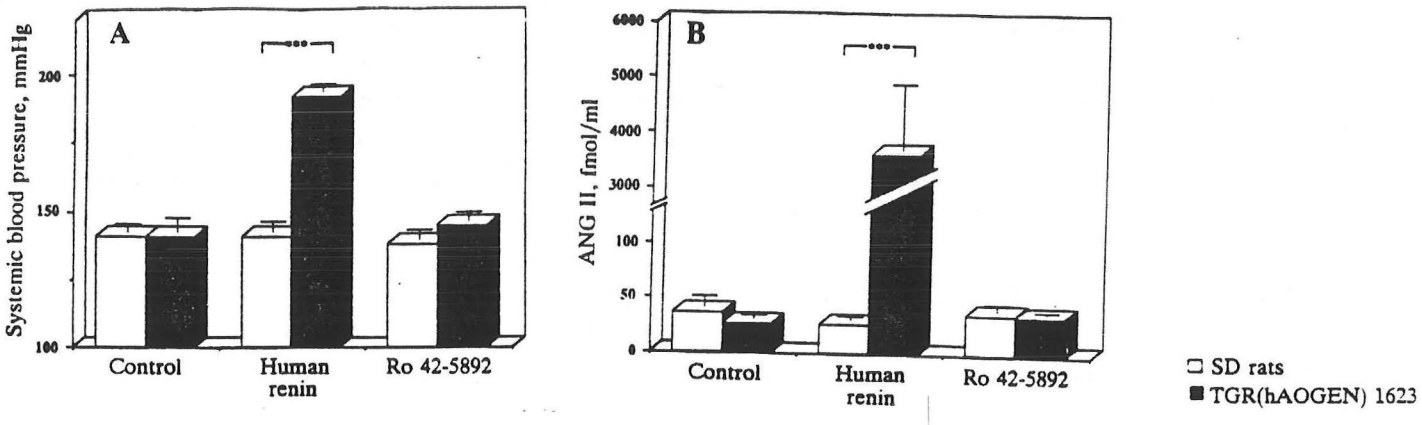
The human angiotensinogen gene was introduced into mice and rats.

Species	Transgene	Tissue Distribution	Phenotype
mouse	human	highest in liver high in kidney low in brain and heart high circulating	normotensive
rat	human	highest in liver high in kidney (proximal tubule), jejunum, brain low in heart and lungs high circulating	normotensive
mouse	rat	highest in liver	normotensive

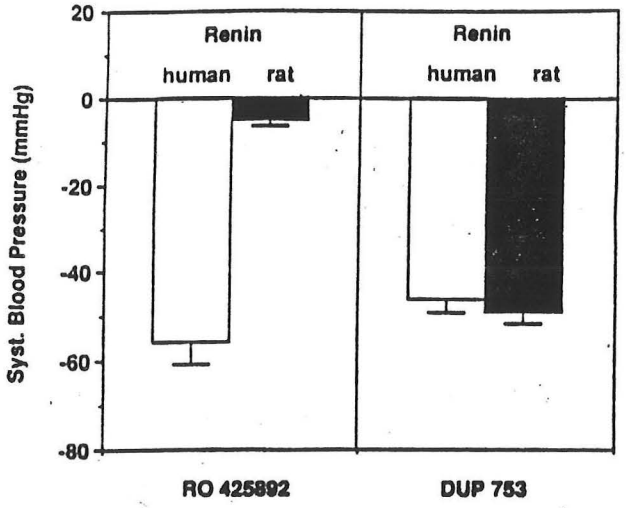
The lack of a hypertensive phenotype in these animals is because of the tight species-specificity of renin as an aspartyl protease. Although AI and AII are very homologous in different species, the rate limiting step of the renin-angiotensin cascade, the renin-substrate reaction, is extremely species-specific. Thus, high levels of human angiotensinogen in rodents will not be cleaved to form the active products AI and AII. If the angiotensinogen transgene is coexpressed with a renin trangene from the same species by cross breeding animals bearing the human angiotensinogen transgene with animals bearing the human renin transgene, the hypertensive phenotype will result (60).

The *in vivo* specificity is illustrated by the rat line carrying the human angiotensinogen transgene TGR9(hAOGEN) (61). The animals were normotensive at

baseline. When human renin was infused, the TGR9(hAOGEN) rats developed high AII levels and hypertension whereas control Spargue-Dawley rats did not respond at all.



Another application with these normotensive lines is in testing acute hypertensive effects of renin and manipulation of renin with pharmacologic agents without using human or primate subjects. This is shown in the following experiments.



Renin infusion in TGR9(hAOGEN)

In transgenic rats carrying the human angiotensinogen gene, a rapid hypertensive response can be induced by infusion of either rat or human renin whereas in Sprague-Dawley rats, human renin does not ellicit hypertension (59). When the human specific renin inhibitor RO425902 is infused, blood pressure was normalized only in human renin-induced hypertension and not rat renin-induced hypertension. In contrast, the non-species specific non-peptide AII antagonist DUP 753 blocks both human and rat AII, thereby lowering blood pressure in both instances.

These experiments showed that transgenic expression of angiotensinogen will lead to hypertension as long as the appropriate renin is present to cleave it. In addition, these animals turns out to be quite valuable in testing agents that interfere with the human renin-substrate reaction using rats as such studies cannot be performed in humans.

Transgenic expression of renin

The human renin gene was first expressed as a trangene in mice by Fukamizu and coworkers (62, 63). Subsequently, rats were used as hosts because they were more suitable for hypertension studies.

Species	Transgene	Tissue Distribution	Phenotype
mouse	human	Highest in kidney (JG cells) low in brain, heart, lung pancreas, spleen, stomach, testis	normotensive
rat	human	Highest in kidney (JG cells) low in lungs and circulation	normotensive hypertensive if bred with rats carrying the angiotensinogen transgene
mouse	mouse	*Ren-1^c, Ren-1^d, Ren-2* isoform study	not measured
mouse	rat	renin and angiotensinogen transgene both in liver	hypertensive
rat	mouse	highest in adrenals suppressed in kidneys	severe low-renin hypertension
rat	human	highest in adrenals suppressed in kidneys	severe low-renin hypertension

I will described the last three models becauss the transgenic expression led to hypertension. In the study of Ohkubo et al. (60), rat renin and angiotensinogen genes were introduced seperately into mouse eggs with *cis*-acting metallothionien promoters fused to part of the native rat promoters (angiotensinogen promoter 1.7 kb; renin promoter 0.8 kb). Both the renin and angiotenogen transgenes were expressed in the liver in a Zn-inducible fashion. When heterozygous lines of each of these transgenic animals were crossed, four genotypes were obtained. The blood pressure measurements are shown.

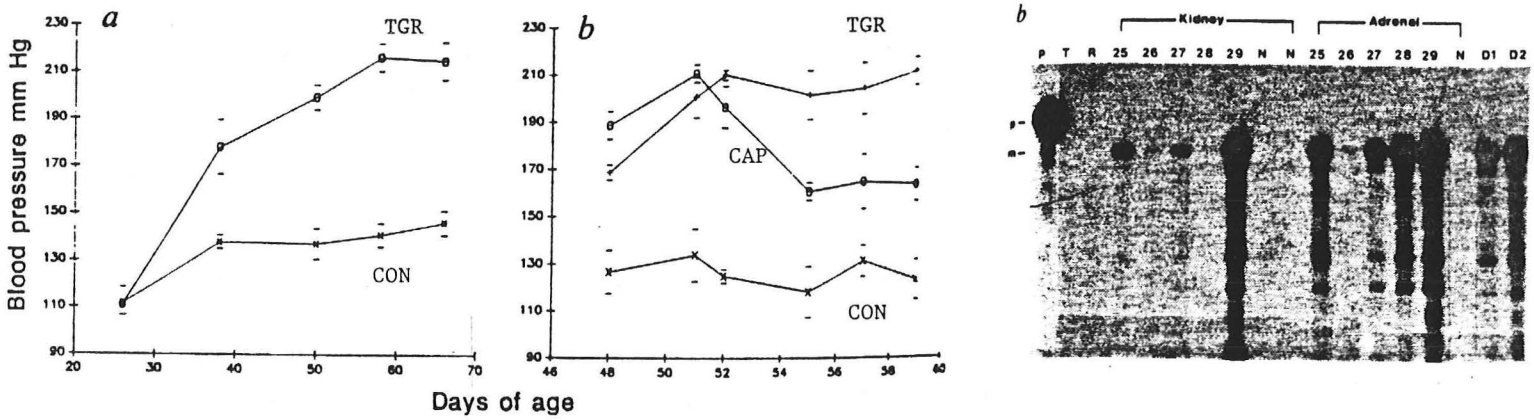
BP readings (mm Hg)		Angiotensinogen gene			
		no		yes	
		male	female	male	female
Renin gene	no	95	107	103	108
Renin gene	yes	95	105	126*	132*

* $p<0.01$

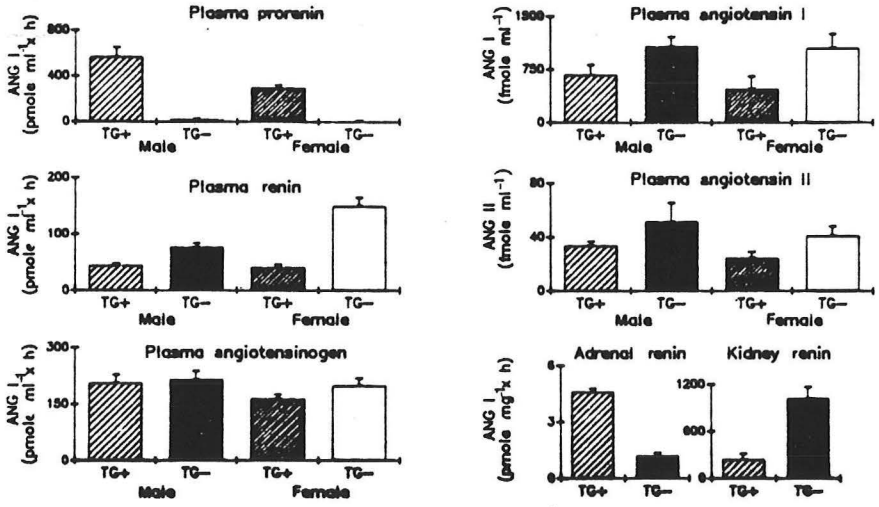
Only the animals harboring both transgenes exhibited hypertension. After 1 month of Zn treatment, the mean BP rose even further but additional of captopril to the drinking water reduced the BP to normal levels. In this example, the interpretation is a little more complex than that of species-specific action of renin. Although mouse angiotensinogen is a poor substrate for rat renin, rat angiotensinogen is a good substrate for mouse renin (64). Therefore one would expect the mice carrying the rat angiotensinogen transgene to be hypertensive. Ohkubo and coworkers argued that the AII generated by this reaction will feed back on the native mouse renin gene expression and suppress it maximally thereby abrogating hypertension. In contrast, in the animals with the double transgenes, the negative feedback mechanism is not functional. Although their data did not prove this hypothesis, this line of reasoning underscores polygenic pathogenesis of hypertension.

A more interesting model are the rats transgenic for mouse renin because rats are more "relevant" hosts as far as human hypertension is concerned. The group at Heidelberg created such a line TGR(mREN2)27 (65). As mentioned before, mouse renin can cleave rat angiotensinogen and infusion of mouse renin into rats leads to a significant increase in blood pressure (66). A linear DNA fragment containing the entire mouse *Ren-2* gene (5.3 kb and 9.5 kb 5'- and 3'-flanking sequences respectively) was introduced into rat eggs. Transgene positive hypertensive founders gave rise to progenies that were hypertensive without exception.

Blood pressure in these animals was elevated by 4 weeks of age reaching a maximum by 9 weeks and was reversed partially by captopril admistration. RNAse protection assays showed that the transgene was expressed in the adrenals and kidneys with adrenal expression far greater than renal.



When tissue and circulating levels of the RAS was examined, renin was only elevated in the adrenals of the transgenic animals with everything else being suppressed, including circulating renin and renal renin.



Although renin was expressed as the transgene, this transgenic model actually resembles patients with low-renin hypertension. A possible pathogenesis is activation of adrenal mineralocorticoids by a local renin-angiotensin system leading to volume expansion and suppression of the endogenous renin angiotensin system. Elevated urinary aldosterone, deoxycorticosterone, corticosterone, and 18-OH corticosterone in these animals, and marked lowering of blood pressure following adrenalectomy support that notion (67, 68). In addition, adrenal renin gene expression is stimulated

by cAMP. Surprisingly, AII which normally inhibits renin release, stimulated renin in the adrenal gland of these animals. The significance of the elevated circulating prorenin which originates from the transgene, is unclear. The existence and importance of local paracrine and autocrine renin-angiotension systems are recognized with increasing frequency, this study uncovers a possible pathogenic mechanism by which local renin-angiotensin systems can alter systemic blod pressure. Another possibility is the paracrine/autocrine renal epithelial renin-angiotensin system (Moe) which can also give rise to a volume dependent low-renin hypertensive state.

SUMMARY

I have described and emphasized the polygenic nature of primary human hypertension and the heavy influence of the hypertensive phenotype by environmental factors and the difficulty these factors imnpose on the study of this disease. To dissect out the etiology of this very heterogenous disease is a formidable task. I summarized some of the approaches that have been used with at least modest success. Significant progress in hypertension research will depend upon recognition of the offending genes to allow the clinician to device more targeted approach to early recognition and specific therapy.

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