

1990-2000: Progress in Determining High Blood Pressure Genes

P B Munroe,*^{PhD}, J Knight,**^{BSc}, M J Caulfield,***^{MD, MRCP}

Abstract

Introduction: This article attempts to summarise the genetic research that has taken place during the past decade to determine the identity of genes causing high blood pressure. **Methods:** Candidate gene studies and genome-wide scanning have been the methods primarily employed, and studies have been performed in both experimental models (rats and mice) and human volunteers (sibling-pairs and case-control). Key studies from the past 10 years are discussed, in addition to the congenic strains. **Results:** Genome-wide scans and candidate gene studies in both rat and man have generated many chromosomal regions and loci involved in blood pressure regulation. However, much work is still required to fine map the large chromosomal regions found in the genome-wide scans and to isolate variants in candidate genes and prove that they are disease-causing. **Conclusions:** It is anticipated that within the next 5 to 10 years at least one blood pressure susceptibility gene will be identified in rat and possibly some in man. It is hoped that the identification of genes controlling blood pressure will enable investigators to determine physiological/biochemical pathways defective in hypertensive patients. This information may then be utilised to identify specific hypertensive phenotypes, to tailor therapy appropriately for patients and hopefully to develop novel therapeutic agents for hypertension.

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Introduction

Essential hypertension and its complications, coronary heart disease, stroke and renal failure are leading causes of morbidity and mortality in western societies.¹ While the aetiology of essential hypertension is not known, it is recognised as being a multifactorial disease caused by both genetic and environmental factors. Evidence for the importance of susceptibility genes in influencing the phenotype comes from epidemiological, twin and adoption studies. It has been proposed that up to 30% of blood pressure variation might be explained by genetic influences and that environmental factors such as diet e.g. salt consumption, weight gain, alcohol intake and stress account for the remainder.²

Determining the genes responsible for blood pressure dysregulation and hypertension is not an easy task. Hypertension is a heterogeneous trait. The researcher does not know the number of genes implicated or how they might interact. Indeed different susceptibility alleles may be responsible for blood pressure regulation in different

individuals or ethnic groups and there may be different environmental triggers in different individuals. All are questions that currently remain unanswered although some progress has been made.

The aim of this review is to look back at the past decade and attempt to summarise the progress that has been made using both experimental models and humans in starting to decipher the genes causing essential hypertension.

Experimental Models

The sequence and function of many genes are the same between human and mammalian genomes and there is also some conservation of synteny—the order of genes on the chromosome. The genetic similarities between species have aided mapping and cloning of many human disease genes, especially for monogenic disorders as it has been possible to derive disease-causing genes from studies of animal models with similar phenotypes.³ The rat is the most widely used animal for hypertension research and genetic analysis in rats like mice is relatively quick and cost-

* Lecturer in Molecular Genetics

** PhD student

*** Professor of Clinical Pharmacology
Department of Clinical Pharmacology

St Bartholomew's and The Royal London School of Medicine and Dentistry, London, United Kingdom

Address for Reprints: Dr Patricia Munroe, Department of Clinical Pharmacology, St Bartholomew's and The Royal London School of Medicine and Dentistry, Charterhouse Square, London EC1M 6BQ, United Kingdom.

E-mail: p.b.munroe@mds.qmw.ac.uk

effective compared to human studies; additionally you can control the environment.⁴ Inbred rat strains have been developed to aid the study of blood pressure regulation. These strains have a limited number of genes influencing the trait and therefore simplify analysis. One of the first hypertensive inbred strains developed was the spontaneously hypertensive rat (SHR).⁵ Since then, many other strains have been established through selective breeding for high blood pressure as well as more specific phenotypes, e.g. stroke-prone SHR (SHR/SP, Table I).

In addition to the part played in genetic research, inbred rats have also been useful in elucidating potential mechanisms controlling blood pressure. For example, kidney transplantation from spontaneously hypertensive rats to normotensive rats demonstrated the pressor effect of the kidney.⁷ These animals have also been utilised for the development and testing of new anti-hypertensive agents.⁸

Mapping of Blood Pressure Loci

Much work in the 1990s has focused on establishing the identity of genes influencing blood pressure in the inbred hypertensive strains. Segregation analysis has been used to establish whether candidate genes are linked to hypertension and to discover new loci using genome-wide screens. The renin gene was one of the first candidate genes studied and Rapp et al⁹ found a variant that co-segregated with high blood pressure in Dahl (s) strain. Another candidate gene studied in rodent models is the cytoskeletal protein, adducin. Two polymorphisms have been found in the adducin gene, one is in the α -subunit and the other in the β -subunit, both differ between the Milan hypertensive strains and Milan normotensive strains and are associated with hypertension.¹⁰ The first two genome screens for blood pressure loci were carried out in 1991 in SHR/SP.^{11,12} Several potential loci were identified, however, only chromosome 10 was significantly linked to high blood pressure in both studies. Many genome screens in the different rat strains have now

been performed (Table II). Various loci have been found, some regions are positive in more than one screen, and others are located in the same region as a candidate gene.

Congenic Strains

To aid identification of blood pressure genes or quantitative trait loci (QTL), it is necessary to reduce the segment of the chromosome in which the gene resides. In the last 3 years, congenic strains have been utilised in rat hypertension research to confirm the presence of the QTL and to narrow the region prior to gene cloning. This would not have been possible without progress that has been made in the identification and mapping of new markers in the rat genome.²³ A congenic strain differs from the original inbred strain by only one section of one chromosome. This technique has been used recently to fine map QTLs on chromosome 1 in both SHR and SHR/SP strains.²⁴⁻²⁷ Congenic strains have also been made for other hypertensive rats, and a study by Garret and colleagues¹⁹ have described the development of four congenic strains with Lewis alleles on a Dahl S background. Speed-congenics has subsequently been utilised to dramatically reduce the time necessary for creation of these strains.²⁸ Congenic strains can also be used to confirm whether there is gene-gene interaction between QTLs by the breeding of double congenics.²⁹ Double congenic strains have two QTLs introgressed from another strain. If the effect on blood pressure of a double congenic is greater than the added effects of two single congenics, there is said to be an epistatic/syntenic relationship between the QTLs.

Mouse models of hypertension also exist and the first genome screens for blood pressure loci have recently been published.³⁰ Markers on chromosomes 10 and 13 showed significant linkage to blood pressure, several other markers showed suggestive linkage. Additional phenotypes were also investigated and chromosome 3 showed evidence of linkage to pulse pressure. None of the mouse loci map to

TABLE I: FIVE COMMONLY USED HYPERTENSIVE RAT STRAINS

Name	Strain of origin	Commonly used control strains	Basis of selective breeding
Spontaneously hypertensive rat (SHR)	Wistar kyoto	Wistar kyoto	Rats with hypertension
Stroke prone SHR (SHR/SP)	Sub-strains of SHR	Wistar kyoto	Rats whose parents had died of stroke
Dahl salt sensitive (Dahl S or SS)	Sprague dawley	Dahl salt resistant	Rats with sensitivity to salt induced hypertension
Sabra hypertensive (SBH)	Sabra outbred	Sabra normotensive (SBN)	Rats that developed hypertension after unilateral nephrectomy and treatment with deoxycorticosterone and sodium chloride
Milan hypertensive strain (MHS)	Wistar	Milan normotensive	Rats with high systolic BP

Information on the rat strains is taken from <http://ratmap.gen.gu.se/ratdocs/RatStraininfo.html>

Further information is available from Handbook of Hypertension, Volume 16 (1994)⁶

TABLE II: MAPPED QTLs FROM GENOME-WIDE SCREENS IN RAT AND MAN

Rat strains	Chromosome	LOD score	Central markers	References
WKY x SHRSP _{Hd}	10	n/a	ACE	Hilbert et al, 1991 ¹¹
	X	n/a	Per-Ha-2/Per-Ha-7	
WKY x SHRSP _{Hd}	10	5.1	ACE	Jacob et al, 1991 ¹²
	18	3.2	Rr1094	
RI BN x SHR	2	n/a	D2N35	Pravenec et al, 1995 ¹³
	4	n/a	Il-6	
	19	n/a	D19Mit7	
BN x SHR	2	3	Mt1pb	Schork et al, 1995 ¹⁴
	2	6.3	Gca	
	2	3	R598	
	4	4.6	Npy2	
	8	5.1	R850	
	16	4.3	R220	
ACI x FHH	1	4.2	Mt1pa	Brown et al, 1996 ¹⁵
WKY x SHRSP _{Gla}	2	3.3	D2Mit14	Clark et al, 1996 ¹⁶
	2	3.1	D2Mit6	
	3	5.6	D3Mgh16	Yagil et al, 1998 ¹⁷
Sabra HR x Sabra HP	1	4.9	D1Mit2, Sa	
	1	4.7	D1Mit1, Cyp450	
	17	3.4	D17Mgh5	
Donryu x SHR	1	4.3	D1Mit3	Innes et al, 1998 ¹⁸
Lewis x Dahl S	5	4.5	Edn2	Garret et al, 1998 ¹⁹
	10	5.5	D10Wox6	
Human populations		<i>P</i> value		
Caucasians [†]	6	<0.0001	D6S1009	Krushkal et al, 1999 ²⁰
	15	<0.0033	D15S652	
	5	<0.0076	D5S1471	
	2	<0.0089	D2S1788	
Chinese [‡]	11		D11S2019	Xu et al, 1999 ²¹
	17		D17S1303	
	3		D3S2387	
	16		D16S3396	
	15		D15S657	

Information on rat QTLs (quantitative trait loci) is taken from Table I in Dominiczak et al (1998).²²

Strain abbreviations:

ACI: AxC9935 Irish; BN: Brown Norway; FHH: fawn-hooded hypertensive; RI: recombinant inbred;

Sabra HP: hypertensive-prone; Sabra HR: hypertensive-resistant; SHR: stroke-prone spontaneously hypertensive;

SHRSP_{Gla}: stroke-prone spontaneously hypertensive Glasgow; SHRSP_{Hd}: stroke-prone spontaneously hypertensive Heidelberg; WKY: Wistar-Kyoto

Gene/marker abbreviations:

ACE: angiotensin converting enzyme; Cyp450: cytochrome p450; Gca: guanylylcyclase A; Il-6: interleukin-6;

Mt1pa: metallothionein 1, pseudogene a; Mt1pb: metallothionein 1, pseudogene b; Npy 2: neuropeptide Y2;

Per-Ha-2/Per-Ha-7: minisatellite bands, remaining symbols represent anonymous microsatellite markers

n/a: LOD score not given in reference

[†] Only loci with significant *P* values from the study are listed, all are linked with systolic blood pressure (SBP).

[‡] No significant *P* values were obtained, the loci with unadjusted *P* < 0.001 are listed. Chromosomes 11, 17, 3 and 16 are linked with SBP, chromosome 15 is linked with diastolic blood pressure.

syntenic regions in the rat, this indicates that investigation of the hypertensive mouse may lead to novel loci being detected.

Transgenic and Knock-out Models

Once there is some evidence that a gene is involved in blood pressure control, either from physiological/pharmacological or from positional cloning projects, there

are methods that can be used to study the mechanism and function of specific genes. Transgenic animals (animals with artificially introduced foreign DNA stably incorporated into their germline) have been widely used in hypertension research for this purpose. The first transgenic rat TGR (mRen-2) 27 was produced and characterised by Mullins et al.³¹ A genomic construct of the mouse Ren-2 gene was injected into the oocytes of normotensive rats; the resultant

transgenic rats developed fulminant hypertension. Other transgenic rats have been established³² and transgenic rats with human genes from the renin-angiotensinogen system (RAS) were important in establishing the species specificity of the human RAS system.³³

Mouse knock-out models of several candidate loci have also been developed, and analysis has confirmed their role in blood pressure regulation.³⁴ In addition, Smithies et al³⁵ have developed an elegant technique, targeted gene duplication and disruption to create mice that can have between 0 and 4 copies of genes, thus allowing investigators to produce mice with varying levels of expression of the candidate gene. Mice have been developed with 0, 1, 2, 3 and 4 copies of the angiotensinogen gene. A correlation was observed between gene copy number and plasma angiotensinogen levels and blood pressure.³⁶

Human Studies

Since the late 1980s, molecular genetic techniques have been used in human hypertension research. Three strategies were primarily employed: linkage analysis in informative families, sibling-pair analysis and population association studies. Early candidate genes were selected because of the role of their protein products in blood pressure regulation or from information gleaned from intermediate phenotypes, i.e., biochemical traits or physiological parameters that tended to co-segregate with hypertension in families. Latterly, candidate genes have been derived from studies of the monogenic forms of hypertension and from analysis of the inbred hypertensive rats.

Candidate Genes and Comparative Mapping

Numerous candidate gene studies have been performed in the 1990s in human essential hypertension. All recognised components of the renin-angiotensin enzymatic cascade have been analysed and the data generated thus far are favourable towards variants of the angiotensinogen gene³⁷⁻³⁹ and suggestive for variants of the angiotensin-type 1 receptor gene^{40,41} predisposing to hypertension. All genes with mutations causing monogenic forms of hypertension have also been studied. There is some support for the epithelial sodium channel gene (Liddle's syndrome⁴²) being involved in the development of hypertension in individuals of African descent.⁴³ Variants of the 11 beta-hydroxysteroid dehydrogenase gene (apparent mineralocorticoid excess⁴⁴) have been described with hypertensive end-stage renal disease⁴⁵ and variants of the aldosterone synthase gene (glucocorticoid remediable aldosteronism, aldosteronism⁴⁶) with hypertension in Caucasian individuals.⁴⁷

Variants in the adducin gene are associated with hypertension in the Milan hypertensive rat (see above).¹⁰ A

Gly460Trp polymorphism in the human homologue has been found to be associated with hypertension in Caucasian individuals.⁴⁸ However, no association has been observed between this variant and hypertension in Japanese individuals.⁴⁹ Adducin is the only candidate gene that has been implicated so far in both experimental and human hypertension. However, there is recent data supporting linkage between a region on the long arm of human chromosome 17 (the region syntenic to rat chromosome 10 where blood pressure loci have been mapped from a genome screen, see above) and hypertension in Caucasian populations.^{50,51} These results suggest that comparative mapping might be a useful accessory in determining high blood pressure genes in humans or at least contribute to our knowledge of the pathways that are involved.

No single gene has yet to be consistently linked or associated with human essential hypertension. These inconsistent results in candidate gene studies can in part be attributed to selection bias of the populations that have been studied and/or insufficient statistical power. Scientists investigating complex diseases are now becoming aware that candidate gene studies must be performed in populations of sufficiently large size to be able to demonstrate differences in genotype frequencies, that more than one polymorphism in a candidate gene requires studying and that excellent phenotypic data are necessary. A recent excellent paper by Corvol and colleagues³⁹ discusses the merits and pitfalls of the candidate gene approach and the lessons that can be learned from studies on two candidate genes, angiotensinogen and the epithelial sodium channel.

The transmission disequilibrium test (TDT)⁵² obviates one of the problems inherent to conventional case/control association tests—hidden population stratification. In this method, preferential transmission of alleles to affected individuals from parents that are heterozygous at the locus in question is tested for. The non-transmitted alleles serve as ethnically matched controls within the family, protecting against problems due to hidden ethnic admixture and selection bias. The statistical power of TDT enables detection of very small levels of genotype relative risk using resources half the size of sibling pair studies.⁵³ This method is fast becoming the method of choice for testing candidate genes and their relationship to disease, however, only recently have candidate genes for hypertension been assessed using TDT analysis. Variants at the angiotensinogen locus (T174M and M235T) have been studied in a Chinese TDT population, however, no association with hypertension was observed.⁵⁴ The TDT method using haplotypes rather than single genotypes of candidate gene markers will be used with increasing frequency in genetic studies in essential hypertension.

Genome-Wide Screens

In 1994, the feasibility of performing genome-wide screens for susceptibility genes for complex diseases was demonstrated.^{55,56} In 1999, the results of the first genome screens for genes regulating blood pressure in man were published.^{20,21} Kruskal and colleagues²⁰ used the discordant sib pair approach⁵⁷ and found four chromosomal regions (2p, 5q, 6q and 15q) linked to systolic blood pressure in their families recruited from Rochester, Minnesota (Table II). Xu and colleagues²¹ identified discordant, highly concordant and low concordant hypotensive and hypertensive Chinese sib pairs. A genome scan did not find any chromosomal regions to reach a 5% genome-wide significance level, but 5 regions gave suggestive linkage to systolic blood pressure (SBP) and one to diastolic blood pressure (DBP) (Table II). Subsequent work from Xu and colleagues⁵⁸ confirmed the existence of a low DBP QTL on the long arm of chromosome 15.

The Future

This review has attempted to summarise the primary research of the past decade into the genetic basis of essential hypertension. In the experimental models of hypertension, numerous blood pressure QTLs have now been mapped, and in the next few years the first rat blood pressure QTL will be identified. Microarrays or similar methods will probably be used for expression profiling and this should help to speed up the cloning procedure.⁵⁹ In human essential hypertension, the results of the first genome scans for blood pressure loci have recently come into the public domain. At this time we do not know if blood pressure QTLs will be syntenic between rat and man, and this is a question that should be answered within the next few years.

A complimentary approach to genome scanning is candidate gene analysis. With the release of human chromosome 22 sequence⁶⁰ and the imminent completion of the human genome sequencing project, many new candidate genes for regulating blood pressure will be identified. This new sequence information and the concerted effort of many labs to identify single nucleotide polymorphisms (SNPs) in both cardiovascular genes^{61,62} and throughout the genome^{63,64} will enable very powerful candidate gene studies and whole-genome linkage disequilibrium mapping to be performed.⁶⁵ These new studies will complement on-going work, and one of the remaining challenges will be to prove that candidate gene polymorphisms are indeed disease causing.

Whether drugs used to treat raised blood pressure tackle the underlying mechanisms is still unclear. As all current drugs have side-effects, targeted therapy based on understanding the genetic mechanisms of blood pressure

elevation in an individual could reduce side-effects and improve the quality of care. It is anticipated that with the cloning of hypertension-causing genes, it will be possible to design therapeutic drugs that are tailored to each individual. Currently, it is hard to predict the type of therapies that will be developed, as this will depend on the number of genes mutated in any individual, their function and tissue distribution and their interaction with environmental stimuli. There is much hope for gene therapy, and for complex diseases such as hypertension, it might be possible if there are major genes influencing the phenotype; however, the mode of delivery and maintaining long-term reduction of blood pressure currently pose problems. Limited gene therapy studies using anti-sense oligonucleotides that switch off the production of message of angiotensinogen have been applied in animal models;⁶⁶ it is too early to say whether this will have any contribution to therapy in humans.

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