STUDIES TO DETERMINE THE PREVALENCE OF CANDIDATE GENE POLYMORPHISM FOR HYPERTENSION AMONG MALAYS

by

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LIST OF SYMBOLS

α	Alpha
β	Beta
γ	Gamma
A	Adenine
C	Cytosine
G	Guanine
T	Thymine
Tm	Melting temperature

LIST OF ABBREVIATION

ACE	Angiotensin-converting enzyme
ADI	Autosomal dominant mode of inheritance
AGT	Angiotensinogen
AME	Apparent mineralocorticoid excess
ANOVA	Analysis of variance
ARI	Autosomal recessive mode of inheritance
Asp	Aspartate
$AGTR_1$	Angiotensin II receptor type 1
$AGTR_2$	Angiotensin II receptor type 2
BMI	Body mass index
bp	Base pair
CAD	Coronary artery disease
CI	Confident interval
cM	Centimorgan
cAmp	Cyclic adenosine monophosphate
CYP11B2	Aldosterone synthase
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
EDHF	Endothelium derived hyperpolarizing factor
EDTA	Disodium ethylenediaminotetra-acetate.$2H_2O$
ENaC	Epithelial sodium channel
eNOS	Endothelial nitric oxide synthase
EtBr	Ethidium bromide

Glu	Glutamate
GRA	Glucocorticoid-remediable hyperaldosteronism
iNOS	Inducible nitric oxide synthase
ISH	International Society of Hypertension
LDL	Low-density lipoprotein
LPC	Lysophosphatidylcholine
Met	Methionine
MLR	Multiple linear regression
mmHg	Millimeter mercury
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
OD	Optical density
PCR	Polymerase chain reaction
PHA	Pseudohypoaldosteronism
PKC	Protein kinase C
PRA	Plasma renin activity
QTL	Qualitative trait loci
RAAS	Renin-angiotensin-aldosterone system
RAS	Renin-angiotensin system
RE	Restriction enzyme
RFLP	Restriction fragment length polymorphisms
RNA	Ribonucleic acid
SBP	Systolic blood pressure
SCNN1B	Gene of beta subunit of epithelial sodium channel
SCNN1G	Gene of gamma subunit of epithelial sodium channel

SEM	Standard error mean
SD	Standard deviation
SHR	Spontaneous hypertensive rat
SLR	Simple linear regression
SNPs	Single nucleotide polymorphisms
SPSS	Statistical Package for Social Science
TAE	Tris-acetate-EDTA
TBE	Tris-borate-EDTA
TDT	Transmission-disequilibrium test
Thr	Threonine
VNTR	Variable number of tandem repeat
WHO	World Health Organization

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LIST OF PRESENTATIONS & PUBLICATION

Presentations

M.G. Dzuzaini, A. Rehman & A. R. A. Rahman
T594M Polymorphism of β-Subunit of the Epithelial Sodium Channel Among Malays; A Preliminary Report
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KAJIAN UNTUK MENENTUKAN FREKUENSI KEJADIAN POLIMORFISME CALON-CALON GEN BAGI HIPERTENSI DI KALANGAN MASYARAKAT MELAYU

ABSTRAK

Hipertensi atau peningkatan tekanan darah arteri adalah merupakan masalah kesihatan umum yang penting dan adalah merupakan faktor risiko yang utama bagi kebanyakkan kecacatan dan kematian kardiovaskular. Tekanan darah dikawalatur oleh pelbagai mekanisme yang melibatkan beberapa lokus genetik dan faktor-faktor persekitaran. "Hipertensi essensial" di kalangan manusia adalah gangguan pelbagai faktor yang disebabkan oleh interaksi di antara kedua-dua faktor genetik dan faktor persekitaran. Penyelidikan berkaitan dengan hipertensi essensial di kalangan manusia berasaskan genetik molekul telahpun dijalankan beberapa tahun yang lalu. Kajian ini bertujuan untuk mengkaji calon-calon gen tertentu yang mungkin menyumbang kepada penghasilan tekanan darah yang tidak normal disebabkan oleh kesannya ke atas sistem kardiovaskular.

Di antara pelbagai calon-calon gen yang tersenarai dan diwartakan hubungkaitnya dengan hipertensi, tiga daripadanya telah dipilih dan perkaitannya dengan kejadian hipertensi dikalangan masyarakat Melayu dikaji di dalam tesis ini. Frekuensi bagi varian polimorfik bagi calon-calon gen yang spesifik ini juga ditentukan. Polimorfisme yang dikaji adalah T594M bagi gen epithelial sodium channel (ENaC), M235T bagi gen angiotensinogen (AGT) dan G894T bagi gen endothelial nitric oxide synthase (eNOS). Dalam kajian ini sejumlah 398 sampel DNA dianalisa daripada seramai 200 orang penghidap hipertensi dan 198 orang subjek kawalan yang mana umur dan jantina mereka telah dipadankan. Kesemua subjek yang terlibat di dalam kajian ini adalah merupakan Melayu tulen bagi tiga generasi berturut-turut.

Taburan frekuensi bagi genotip M235T di dalam populasi adalah 3.5% bagi homozigos liar (MM), 30.4% bagi heterozigos (MT) dan 66.1% bagi homozigos mutan (TT). Frekuensi bagi genotip TT adalah sebanyak 63.5% bagi hipertensi dan 68.7% bagi kawalan. Frekuensi bagi alel M235 dan T235 adalah 20.0% dan 80.0% dikalangan penghidap hipertensi dan 17.4% dan 82.6% dikalangan subjek kawalan. Tiada perbezaan yang signifikan dapat dicerap pada frekuensi genotip (χ^2=1.30, p=0.52) dan alel (χ^2=0.87, p=0.35) di antara kedua-dua kumpulan kajian. Risiko bandingan bagi hipertensi adalah sebanyak 1.01 bagi subjek yang membawa genotip TT dan sebanyak 0.84 bagi individu yang memiliki alel T bagi varian ini.

Sebaliknya, taburan genotip bagi G894T adalah 74.1% bagi homozigos liar (GG), 24.6% bagi heterozigos (GT) dan 1.3% bagi homozigos mutan (TT). Hanya lima individu daripada keseluruhan subjek kajian didapati membawa genotip mutan. Bila dikategorikan keseluruhan populasi kajian kepada dua kumpulan, didapati frekuensi GG, GT dan TT adalah 72.0%, 27.0% dan 1.0% bagi hipertensi dan 76.3%, 22.2% dan 1.5% bagi kawalan. Di dalam kajian ini, secara amnya frekuensi bagi alel G894 dan T894 adalah 85.5% dan 14.5% dikalangan penghidap hipertensi dan 87.4% dan 12.6% dikalangan subjek kawalan. Penganalisaan mengunakan analisis chi-kuasadua (χ^2) mendapati tiada perbezaan yang signifikan dicerap pada frekuensi genotip (χ^2=0.94, p=0.33) dan alel (χ^2=0.60, p=0.44) di antara kedua-dua kumpulan kajian. Risiko bandingan bagi hipertensi adalah sebanyak 1.25 bagi subjek yang membawa genotip TT dan sebanyak 1.17 bagi individu yang memiliki alel T bagi varian ini.

Di dalam menentukan frekuensi polimorfisme T594M bagi β-ENaC didapati bahawa keseluruhan subjek kajian ini adalah merupakan jenis liar bagi varian ini. Tiada seorang pun dikalangan subjek kajian yang membawa genotip homozigos mutan ataupun heterozigos. Di andaikan bahawa varian T594M bagi β-ENaC adalah jarang terdapat dikalangan populasi Melayu.

Analisis statistik yang dilakukan menggunakan Multiple Linear Regression (MLR) menunjukkan bahawa tekanan darah sistolik dan diastolik tidak berkolerasi secara signifikan dengan genotip β-ENaC T594M, AGT M235T dan eNOS G894T di dalam kedua-dua kumpulan kajian. Genotip bagi setiap varian bukan merupakan faktor penentu bagi kedua-dua tekanan darah sistolik dan diastolik di dalam keseluruhan populasi kajian. Maka dapat disimpulkan bahawa varian polimorfik bagi calon-calon gen kajian bukanlah merupakan faktor penyebab utama bagi kejadian hipertensi di dalam populasi Melayu.

Katakunci: Hipertensi, Melayu, gen angiotensinogen, gen epithelial sodium channel, gen endothelial nitric oxide synthase, polimorfisme gen

STUDIES TO DETERMINE THE PREVALENCE OF CANDIDATE GENE POLYMORPHISM FOR HYPERTENSION AMONG MALAYS

ABSTRACT

Hypertension or elevated arterial blood pressure is a substantial public health problem and is a major risk factor for many common causes of cardiovascular morbidity and mortality. Blood pressure is regulated by a variety of mechanisms that involve the products of several genetic loci and a number of environmental factors. "Human essential hypertension" is a multifactorial disorder that is complicated by the interaction of both genetic and an environmental factors. Research on the molecular genetics of human essential hypertension developed several years ago. One approach is to study candidate genes that may contribute to abnormal blood pressure because of their known effect on the cardiovascular system.

Among the listed of candidate genes published for their known associations with hypertension, three of them were selected and their association with hypertension in the Malays was investigated in this thesis. The prevalence of these polymorphic variants within the specific candidate genes in the population was also determined. Polymorphisms studied were the T594M of the epithelial sodium channel (ENaC) gene, the M235T of the angiotensinogen (AGT) gene and the G894T of the endothelial nitric oxide synthase (eNOS) gene. A total of 398 DNA samples from 200 hypertensives and 198 age- and sex- matched controls were analyzed in this genetic polymorphism study. Subjects recruited in this study were pure Malay for three generations.

The frequency distribution of the M235T genotypes in the population was 3.5% for homozygous wild-type (MM), 30.4% for heterozygous (MT) and 66.1% for homozygous mutant (TT). The frequency of the TT genotype was 63.5% in hypertensives and 68.7% in controls, respectively. The frequencies of the M235 and T235 alleles were 20.0% and 80.0% among the hypertensives and 17.4% and 82.6% among the controls. No significant difference was observed in genotype (χ^2=1.30, p=0.52) and allele (χ^2=0.87, p=0.35) frequencies among the two study group. The odds ratio for hypertension is 1.01 for subjects carrying the TT genotype and 0.84 for those having T allele of the variant.

In contrast, the distribution of genotypes for G894T was 74.1% for homozygous wild-type (GG), 24.6% for heterozygous (GT) and 1.3% for homozygous mutant (TT), respectively. Only five individuals from the overall study subjects were found to be carrying the mutant genotype. When categorized into two groups, it was found that the frequency of GG, GT and TT were 72.0%, 27.0% and 1.0% in hypertensives and 76.3%, 22.2% and 1.5% in controls. In this study, the frequencies of the G894 and T894 alleles were 85.5% and 14.5% among the hypertensives and 87.4% and 12.6% among the controls, respectively. Using chi-square analysis, no significant difference was observed in genotype (χ^2=0.94, p=0.33) and allele (χ^2=0.60, p=0.44) frequencies between both study groups. The odds ratio for hypertension is 1.25 for subjects carrying the TT genotype and 1.17 for those having allele T of the variants.

In determining the prevalence of T594M polymorphism of the β-ENaC, it was found that the overall study subjects are wild-type for this variant. None of the study subjects carried a homozygous mutant or heterozygous genotype. It is likely that T594M variant of the β-ENaC gene is rare among Malay population.

Statistical analysis done using Multiple Linear Regression (MLR) shows that systolic and diastolic blood pressure were not correlated significantly with β-ENaC T594M, AGT M235T and eNOS G894T genotype in both study groups. The genotype of each variant was not a predictor for both systolic and diastolic blood pressure in the overall study population. Hence, polymorphic variant of studied candidate genes is not likely to be a major factor causing hypertension in Malay population.

Keywords: Hypertension, Malays, angiotensinogen gene, epithelial sodium channel gene, endothelial nitric oxide synthase gene, gene polymorphism.

CHAPTER 1
INTRODUCTION

1.1 Hypertension

Hypertension or elevated arterial blood pressure is a substantial public health problem and is a major risk factor for many common causes of morbidity and mortality including stroke, myocardial infarction, congestive heart failure and end stage renal disease (Kannel, 2000, Mosterd *et al.*, 1999). Despite the important role of hypertension as a cause of disease, its pathogenesis remains largely unknown. Substantial efforts have been done to defining the pathogenesis of blood pressure variation. Epidemiologic studies have shown the impact of a variety of factors including age, gender and body mass index (Stanton *et al.*, 1982). Diet has also been implicated with salt, potassium and calcium suggested as important factor (Appel *et al.*, 1997). How these factors influence physiology to alter blood pressure has been the subject of extensive investigation.

The difficulty in defining the causes of hypertension from physiologic studies alone has motivated the application of genetic approaches to hypertension. Identification of genes underlying blood pressure variation has the capacity to define primary physiologic mechanisms underlying this trait, thereby clarifying disease pathogenesis, identifying pathways and targets for therapeutic intervention, providing opportunity for preclinical diagnosis and allowing treatment tailored to underlying abnormalities in individual patients (Lifton *et al.*, 2001)

In year 1999, the World Health Organization and the International Society of Hypertension (WHO-ISH) Guidelines Committee has published guidelines for management of chronically elevated blood pressure based on evidence from epidemiological studies and clinical trials. According to these guidelines, hypertension is defined as a systolic blood pressure (SBP) of 140 mmHg or higher and/or a diastolic blood pressure (DBP) of 90 mmHg or greater (Table 1.1). The WHO-ISH also suggested few important areas of research to be focused in future. These include genetic studies on hypertension.

In developed and developing countries alike, essential hypertension affects 25-35% of the adult population, and up to 60-70% of those beyond the seventh decade of life (Staessen *et al.*, 2003). Large population surveys in many countries (Burt *et al.*, 1995, Nissinen *et al.*, 1988) showed its prevalence varied from one to over 30 percent. A similar high prevalence was found in a previous national survey in 1986 as well as in smaller survey (Kandiah *et al.*, 1980, Osman *et al.*, 1984) in Malaysia. The prevalence of hypertension in Malaysia was found to be between 14.0 to 24.1% (Ministry of Health, 1999). Results from the National Health and Morbidity Survey (1996) found a prevalence of 33% or an estimated of 2.6 million Malaysian adults aged 30 or older had hypertension (Table 1.2) (Lim and Morad, 2004).

Table 1.1: Definitions and classification of blood pressure levels (mmHg) according to JNC VI

Category	Boundaries	
	Systolic	**Diastolic**
Optimal	< 120	< 80
Normal	< 130	< 85
High-normal	130 – 139	85 – 89
Grade 1 hypertension (mild)	140 – 159	90 – 99
Subgroup : borderline	140 – 149	90 – 94
Grade 2 hypertension (moderate)	160 – 179	100 – 109
Grade 3 hypertension (severe)	≥ 180	≥ 110
Isolated systolic hypertension	≥ 140	< 90
Subgroup : borderline	140 – 149	< 90

Table 1.2: Prevalence of hypertension in Malaysian adult population

Ethnicity	Sex	N	Prevalence % (SE)	Age adjusted * prevalence % (SE)	Estimated population (SE)
All	Both	21,391	32.9 (0.5)	32.9 (0.4)	2,577,044 (56200)
	Men	10,003	31.9 (0.6)	32.1 (0.5)	1,260,209 (33598)
	Women	11,388	33.9 (0.6)	33.5 (0.5)	1,316,834 (31264)
Malay	Both	9,656	33.5 (0.6)	33.5 (0.5)	1,138,790 (34917)
	Men	4,502	29.9 (0.8)	30.0 (0.7)	496,390 (18062)
	Women	5,154	37.1 (0.8)	36.9 (0.7)	642,400 (20792)
Chinese	Both	5,978	33.1 (0.8)	31.1 (0.6)	791,090 (32277)
	Men	2,746	35.2 (1.1)	34.0 (1.0)	420,028 (18848)
	Women	3,232	30.9 (1.0)	28.2 (0.8)	371,062 (17080)
Indian	Both	1,467	30.8 (1.3)	31.7 (1.3)	186,257 (14206)
	Men	679	34.9 (1.8)	35.5 (1.9)	103,965 (8597)
	Women	788	26.9 (1.7)	27.9 (1.5)	82,292 (7156)
Other indigenous	Both	3,194	34.3 (1.0)	34.8 (0.9)	237,413 (12338)
	Men	1,482	32.2 (1.3)	32.8 (1.3)	112,073 (6268)
	Women	1,712	36.4 (1.3)	36.8 (1.2)	125,340 (6983)

* Age-adjusted to the 1996 Malaysian population

Adapted from : (Lim and Morad, 2004)

1.2 Genetics of hypertension

'Hypertension' is an arbitrary definition and not a quantitative trait that appears relatively late in life. When discussing about the genes involved in hypertension, nothing much is known about their mode of transmission, their quantitative effects on blood pressure, their interaction with other genes, or their modulation by environmental factors. Parameters such as ethnicity and body weight increase the genetic heterogeneity and the difficulty of replication from one study to another (Corvol *et al.*, 1999).

There is substantial evidence for genetic influence on blood pressure. Twin studies show greater concordance of blood pressures in monozygotic than dizygotic twins (Feinleib *et al.*, 1977) and population studies demonstrate greater similarity of blood pressure within families than between families (Longini *et al.*, 1984). This familial aggregation is not simply attributable to share environmental effects since adoption studies show greater concordance of blood pressure among biological siblings than adoptive siblings living in the same household (Biron *et al.*, 1976, Perusse *et al.*, 1989). Finally, that single genes can impart large effects on blood pressure is demonstrated by rare Mendelian forms of high and low blood pressure (Lifton, 1996).

Despite this evidence of a large effect of inheritance on blood pressure, these same studies suggest that blood pressure is commonly multifactorial in determination since *(i)* blood pressure does not typically segregate in families in a fashion consistent with Mendelian transmission and *(ii)* a variety of other factors such as salt intake, age, gender and body mass can influence blood

pressure. It is popular to presume that the blood pressure in individual patients is due to the combined effects of variation at a number of blood pressure-determining loci, environmental factors and demographic factors (Figure 1.1) (Lifton, 1995).

Hypertension has been termed by geneticists as 'a polygenic disorder that is a result of manifested phenotype that is substantially affected by the environment' (Hamet, 1996). Based on the mode of inheritance, hypertension can be classified as either Mendelian hypertension (monogenic forms of hypertension) or essential hypertension (polygenic forms of hypertension). Mendelian forms of hypertension result from a single defective gene, and individuals harboring such mutated genes transmit them in a dominant or recessive manner. In contrast, essential hypertension occurs as a consequence of a complex interplay of a number of genetic alterations and environmental factors, and therefore does not follow a clear pattern of inheritance, but exhibits familial aggregation of cases.

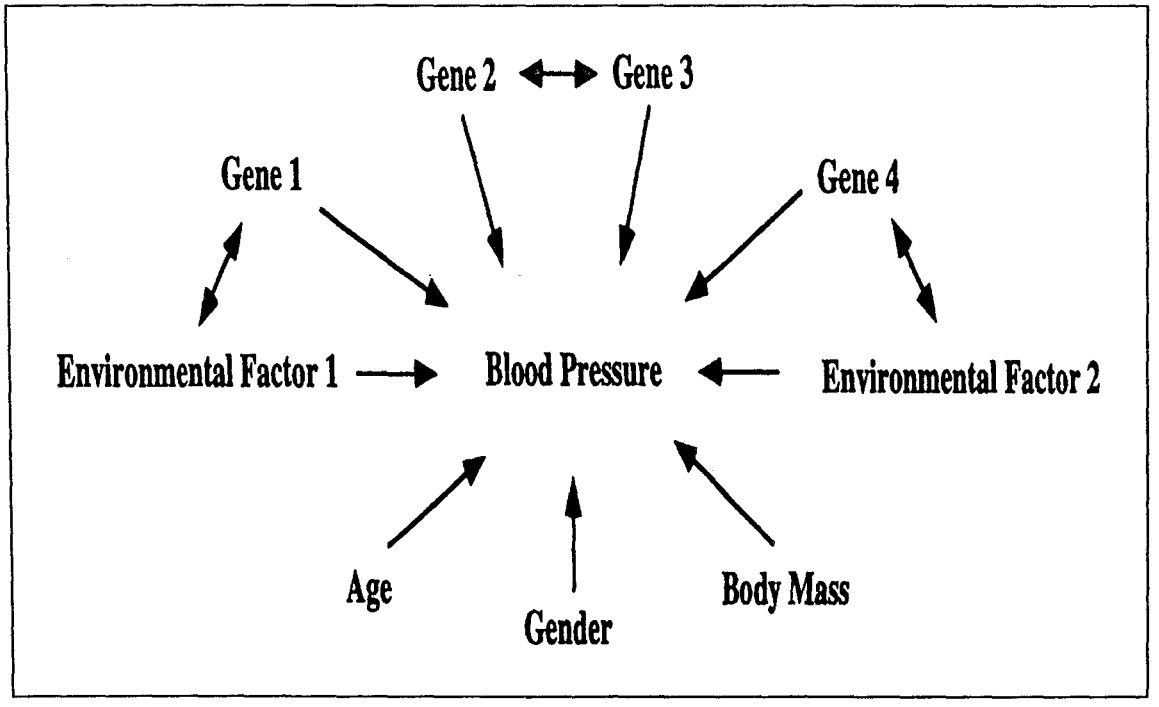

Figure 1.1: Multifactorial model of blood pressure determination, demonstrating the potential influence of genes, environmental factors and demographic factors on blood pressure.

(The potential interaction of these determining factors is represented by arrows linking different determinants).

Adapted from : (Lifton, 1995)

1.2.1 Monogenic forms of hypertension

Considerable progress has been made during the past few years towards unraveling the molecular genetics of some rare, or extremely rare, monogenic forms of hypertension. Although it is widely accepted that blood pressure is a polygenic trait, it is recognized that a small proportion of familial forms of hypertension are inherited as single gene disorders. Molecular genetic studies have identified mutations in eight genes that cause Mendelian forms of hypertension and nine genes that cause Mendelian forms of hypotension in humans (Lifton *et al.*, 2001). These genes typically impart very large effects on blood pressure (Table 1.3). However, lessons learned from the monogenic forms of hypertension have highlighted what the clinical benefits could be if susceptibility genes were to be identified also for essential hypertension.

Table 1.3: Mendelian forms of blood pressure dysregulation

Syndrome	Chromosome	Mode of inheritance *	Effect on blood pressure
Glucocorticoid-remediable aldosteronism (GRA)	8q22	ADI	Increased
Apparent mineralocorticoid excess (AME)	16q22	ARI	Increased
Hypertension exacerbated by pregnancy	4q31.1	ADI	Increased
Hypertension with brachydactyly	12p11.2-p12.2	ADI	Increased
Steroid 11β-hydroxylase deficiency	8q21	ARI	Increased
Steroid 17α-hydroxylase deficiency	10q24.3	ARI	Increased
Gordon's syndrome	1q31-q42 12p13 17q21-q22	ADI	Increased
Liddle's syndrome	16p12-p13	ADI	Increased
Gitelman's syndrome	16q13	ARI	Decreased
Bartter's syndrome	15q15-q21 11q24 1q36	ARI	Decreased
Aldosterone synthase deficiency	8p21	ARI	Decreased
Steroid 21-hydroxylase deficiency	6p21.3	ARI	Decreased

* ADI - autosomal dominant mode of inheritance;

ARI - autosomal recessive mode of inheritance

1.2.2 Essential hypertension

Essential hypertension, or hypertension of unknown cause, accounts for more than 90% of cases of hypertension. It refers to a lasting increase in blood pressure with heterogeneous genetic and environmental causes (Staessen *et al.*, 2003). Evidence suggests that genes may contribute to 30% of the variation of blood pressure. However, the number of genes involved or the model of interaction with other genes or environmental risk factors is unknown (Williams *et al.*, 1991). The multifactorial, polygenic nature and complex etiology of essential hypertension has made the study of this disease difficult. The resulting phenotypes can be modulated by various environmental factors, thereby altering the severity of blood pressure elevation and the timing of hypertension onset.

The prevalence of essential hypertension is 3.8 times greater in individuals with a positive family history of hypertension, suggesting that hypertension has a genetic component (Su and Menon, 2001). There is less evidence for heritability in older age groups (Hunt *et al.*, 1986). Although familial aggregation of essential hypertension is well established, inheritance does not follow a simple Mendelian pattern. A large proportion of the phenotypic variation in blood pressure appears to be inherited as polygenic trait. Therefore, the expression of hypertension in any individuals is likely to be the result of a number of genetic alternations.

The effects of individual genes can be independent and additive, or can be more complex, characterized by phenomena such as epistasis (i.e. the interactive effect of multiple genes on a phenotype) or pleiotropy (i.e. the

simultaneous effects of a single gene on multiple phenotypes) (Schork *et al.*, 1996). Furthermore, environmental factors affect the expression of gene function (Hamet, 1996). Therefore, it is difficult to demonstrate a statistically significant correlation between occurrence of a particular gene or allelic variant and the hypertensive phenotype in a patient population.

1.2.2.1 Identification of Susceptibility Loci

Discovering the major susceptibility locus can be the key to advances in understanding the causes of a disease (Gambaro *et al.*, 2000). Owing to these uncertainties, it is difficult to determine the optimal approach to identification of susceptibility loci affecting blood pressure in humans. A number of alternative approaches can be considered at present. The identification of susceptibility loci can be pursued by different approaches: in families, either by segregation analysis, which can suggest the existence of a major locus and at least in part define its properties; or by linkage analysis of candidate genes or markers to localize a gene, followed by positional cloning; and in the general population by association studies in homogeneous populations.

Segregation and linkage analysis face difficulties with non-Mendelian conditions; indeed the former is very sensitive to biases, whereas the latter requires the specification of a precise genetic model, including disease gene frequency and the penetrance of each genotype. Therefore, population association studies are mostly used in identification of susceptibility loci (Gambaro *et al.*, 2000).

1.2.2.2 Association and linkage studies in essential hypertension

The study of human essential hypertension can be carried out using two basic molecular genetic approaches, association and linkage analysis (Davidson *et al.*, 1995, Lander and Schork, 1994, Luft, 1998). Both approaches require the detection of polymorphic markers such as bi-allelic restriction fragment length polymorphisms and variable nucleotide tandem repeats. These two approaches are not mutually exclusive, but can be merged into a single analytical method, such as transmission or disequilibrium test (TDT) (Spielman and Ewens, 1996). Each has advantages and disadvantages depending on situations tested.

Association studies test whether a particular allele occurs at higher frequency among affected when compared to unaffected individuals and is generally restricted to a genomic region or a variant being tested. This approach is heavily dependent on matching affected with unaffected subjects. If a significant association emerges, the polymorphism itself is either in the susceptibility locus or in linkage disequilibrium with the susceptibility locus. In both cases, it should enable detection of people at risk of developing a certain disease or complications (Gambaro *et al.*, 2000).

Genome-wide linkage studies for complex traits such as essential hypertension are problematic and require careful study design. This is because each variant individually contributes only modestly to risk of disease. A positive result using a linkage approach which generally tests related individuals, will implicate a region of genomic DNA containing a large number of genes, of which the causative gene for a particular disease will still have to be identified

(Lee *et al.*, 2000). Improved techniques in genome-wide linkage analysis have enabled a search for genes that contribute to the development of essential hypertension in the population (Oparil *et al.*, 2003).

The above association and linkage studies have broken substantial ground and have yielded highly promising results suggesting a number of candidate genes and genomic regions that need further investigation as contributors to blood pressure variation. At the same time, some inconsistencies in the results and difficulties of replication in some studies illustrate a number of potential problems inherent in most genetic epidemiological studies of complex disorders (Timberlake *et al.*, 2001).

1.2.2.3 Insights from Animal Models

An alternative approach of studying hypertension is based on the co-segregation of phenotypes using experimental crosses derived from genetically hypertensive and control rat strains (Lee *et al.*, 2000). Rat models of hypertension have been developed and studied over many generations. Their complete genetic heterogeneity, large number of progeny, the ability to produce large genetic crosses and to provide tight control over environmental influences permit the dissection of complex traits such as hypertension (Table 1.4)(Lander and Schork, 1994, Smirk and Hall, 1958).

Genetic models of experimental hypertension developed to approximate the pathogenesis of human hypertension include the spontaneously hypertensive rat (SHR), the SHR-stroke-prone strain, Lyon hypertensive and normotensive rat strains, Milan hypertensive and normotensive rat strains and Dahl salt sensitive rat strains (Bohr and Dominiczak, 1991). These models differ in genetics, cellular alternations and neurohumoral mechanisms. However, they all share the same feature of spontaneous development of hypertension under appropriate conditions. The two most commonly studied are the SHR and Dahl sensitive and resistant strains.

Studies of gene interactions can be performed in these animal models, and the use of congenic animals constitutes a power tool to isolate and test specific chromosome regions in various genetic backgrounds (Kato, 2002). In addition, genes predisposing to hypertension in animal models may also be involved in the etiology of human hypertension, and hence the regions of

homology, or more directly the genes implicated in animal models, can be considered candidate regions or genes to be explore in human disease.

As with any experimental model, there has been intense debate over the applicability of rat models to human hypertension. Rat chromosome 10 contains two blood pressure quantitative trait loci (QTL) (Hilbert *et al.*, 1991, Jacob *et al.*, 1991) and is syntenic to human chromosome 17. Linkage of markers with hypertension has been found on human chromosome 17 (Baima *et al.*, 1999, Julier *et al.*, 1997). These studies provide some support for comparative mapping as a method of identifying candidate regions for the human disorder.

Table 1.4: Genetic analysis of hypertension in inbred rats and humans

	Inbred rats	Humans
Expected number of susceptibility genes	Several genes with major genetic effects	unknown
Heritability of hypertension	~ 60%	30 – 40%
Environmental factors	Controllable	Uncontrollable
Onset of hypertension	Early age	Advances age
Genetic homogeneity	High (inbred)	Low (outbred)
Reproducibility of BP measurement	Relatively good	Relatively poor
Analytical approaches	QTL mapping in experimental crosses	Linkage analysis (eg. affected sib-pairs)
	Development of congenic strains	Association study (unrelated subjects)

QTL – quantitative trait loci

Adapted from : (Kato, 2002)

1.3 Genome-wide Scan

Another major strategy for identifying loci contributing to a complex trait is genome-wide scanning. This involves genotyping family members using highly polymorphic markers spaced at regular interval (10-30 cM) across the entire genome. Each of the markers is then tested for linkage with the trait or disease of interest to identify genomic regions that may harbor trait-influencing loci (Timberlake *et al.*, 2001). There are now many publications describing the results of genome-wide scans for genes controlling blood pressure (Table 1.5). The majority have reported numerous chromosomal regions with suggestive evidence of linkage (Garcia *et al.*, 2003, Samani, 2003). These studies lay important foundations for large-scale human hypertension research and suggest a number of promising genomic regions that may contain blood pressure loci.

Genome-wide scans for hypertension do not presume any prior knowledge of the mechanisms underlying blood pressure regulation. Genome-wide scanning followed by positional cloning has recently proven successful in identifying disease susceptibility genes for two other complex diseases; type 2 diabetes mellitus and Crohn's disease (Horikawa *et al.*, 2000, Hugot *et al.*, 2001). So far, no primary hypertension susceptibility gene has been identified by this method but a number of promising chromosomal regions showing evidence of linkage to hypertension or blood pressure variation have been identified (Krushkal *et al.*, 1999, Levy *et al.*, 2000, Xu *et al.*, 1999).

Each genome scan reported differs in numbers, ethnicity, family types, design and the phenotyping strategy, where there are studies using blood pressure as a quantitative phenotype while others using hypertension (Mein *et al.*, 2004). The use of different markers, study designs and poor precision with linkage data make it difficult to know if the studies have found the same gene. The inconsistency of results among these human genome-wide studies may reflect differences in the ethnic composition and selection criteria for the study population. Also, these results may underscore the heterogeneous and polygenic nature of hypertension (Timberlake *et al.*, 2001).

In future, a hybrid approach has been suggested: genome-wide scans on one hand is to identify new susceptibility genes and disease-causing haplotype, and on the other hand will provide detailed studies of overall sequence variation of candidate genes in order to understand the underlying functional mechanism. The challenge now is to identify which of these loci are genuine and it will be the results of fine mapping or grid tightening in the first instance that will yield this information (Mein *et al.*, 2004).

Table 1.5: Genome-wide scan for blood pressure loci in human

Author	Chromosome	Markers	Potential candidate genes
Krushkal *et al.*	6	D6S1009	Estrogen receptor
(1999)	15	D15S652	Aminopeptidase
	5	D5S1471	Alpha 1B andrenoergic receptor
	2	D2S1788	Sodium-calcium exchanger 1
Xu *et al.* (1999)	11	D11S2019	Angiotensin receptor like 1
	17	D17S1303	Pseudohypoaldosteronism type II
	3	D3S2387	ATPase Ca^{2+} transporting membrane
	16	D16S3396	Thiazide-sensitive NaCl co-transport
	15	D15S203	Insulin-like growth factor 1
	15	D15S657	Cholinergic receptor

Adapted partially from : (Timberlake et al., 2001)

1.4 Specific candidate genes in hypertension

Despite major advances during the past few years in biotechnology and bioinformatics, the classical candidate gene approach continues to be the most prevalent approach in the search for the genetic basis of hypertension. The candidate gene approach typically compares the prevalence of hypertension or the level of blood pressure among individuals of contrasting genotypes at candidate loci in pathways known to be involved in blood pressure regulation (Oparil *et al.*, 2003). The contribution of candidate genes to the pathophysiology of hypertension is continuing to be tested mostly using association studies in humans, and also using physiological and pharmacological interventional studies in animal models and in genetically modified strains (Yagil and Yagil, 2005).

Candidate genes are selected because of their logical or already established effect on cardiovascular and renal function, and on the basis of the known pathophysiology of essential hypertension. Thus, candidate genes for essential hypertension have been suggested by the elucidation of the molecular genetics of rare monogenic forms of hypertension, such as Liddle's syndrome (Shimkets *et al.*, 1994). A variety of candidate genes have been investigated, including loci involving the renin-angiotensin-aldosterone system (RAAS), epithelial sodium channel (ENaC), the renal kallikrein-kinin system, α-adducin and others involving lipoprotein metabolism, hormone receptors, and growth factors (Figure 1.2).

The study of candidate genes, which assumes that a given gene or a group of genes involved in a specific function might contribute to blood pressure variation, has resulted in conflicting results (Lee *et al.*, 2000). Even though the candidate gene approach is widespread and the list of candidate genes for hypertension is increasing, there is still a problem with this strategy in the mission to unwind the complexity of hypertension. The initiative and efforts invested in the candidate gene approach appear to have been sporadic and uncoordinated, resulting in a long list of candidate genes that are not necessarily related to one another (Table 1.6). A more favorable view of the candidate gene approach is that the genetic basis of hypertension is analogous to a huge puzzle and the candidate genes are the individual pieces in that puzzle (Yagil and Yagil, 2005).

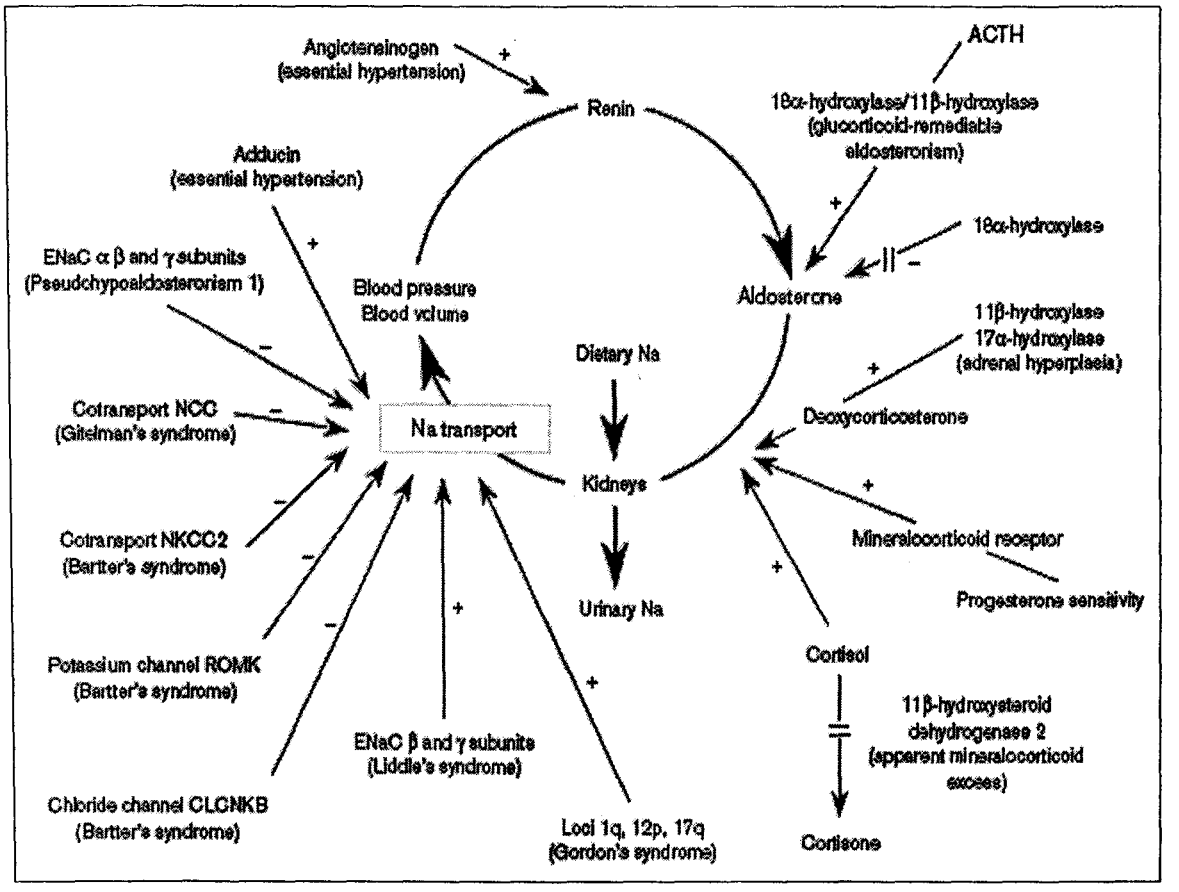

Figure 1.2: Mutations and polymorphisms altering blood pressure in humans.

(All the genes involved directly or indirectly in the control of renal sodium reabsorption. The mutations have been found in monogenic human diseases that cause disorders of blood pressure regulation).

Adapted from : (Warnock, 2001)

Table 1.6: Partial list of candidate genes tested for an association with hypertension

Candidate gene	Abbreviation
Renin-angiotensin-aldosterone system related	RAAS
1. Angiotensinogen	AGT
2. Angiotensin-converting enzyme	ACE
3. Angiotensin II receptor 1	AT_1R
4. Aldosterone synthase	CYP11B2
Non-RAAS related	
1. Peroxisome proliferator-activated receptor γ	PPARγ
2. Insulin receptor substrate 1	IRS-1
3. Serine-threonine kinase with no lysine (K)1	WNK1
4. Serine-threonine kinase with no lysine (K)4	WNK4
5. Lipoprotein lipase	LPL
6. β3 subunit of G proteins	GNB3
7. Hepatocyte growth factor	HGF
8. Solute carrier family 4, member 4	SLC4A4
α_{2B}-Adrenoreceptor	
1. Potassium large conductance calcium activated channel, subfamily M, beta member 1	KCNMB1
Thromboxane	
1. Solute carrier family 6 (neurotransmitter transporter), member 18	XT2
2. Endothelin receptor type A	EDNRA
3. FK506-binding protein 1B	FKP1B
4. Adenosine receptor A1	ADORA1
5. Adenosine receptor A2	ADORA2
6. Epithelial sodium channel β1 subunit	SCNN1B
7. Epithelial sodium channel γ1 subunit	SCNN1G
Adducin	
1. G protein-coupled receptor kinase 2	GRK4
2. Protein tyrosine phosphatase, non receptor type 1	PTP1B
3. 1 Hydroxysteroid dehydrogenase-1, δ^5-3-β	HSD3B
4. Uncoupling protein 2	UCP2
5. Insulin-like growth factor 1 receptor	IGFIR
6. Myocyte specific enhancer factor 2A	MEF2A
7. Paired basic amino acid-cleaving enzyme 4	PACE4

1.4.1 Epithelial Sodium Channel (ENaC)

Among many factors affecting the blood pressure variation, the kidney plays an important role in the long-term regulation of sodium balance, blood volume, and blood pressure via pressure diuresis and pressure natriuresis (Guyton *et al.*, 1972, Liard *et al.*, 1974). It has been known for a long time that the kidneys play a vital role in the long-term control of blood pressure (Guyton *et al.*, 1994), and recently this concept has been reinforced by the identification of the molecular defects in monogenic forms of hypertension (Lifton, 1996). The kidney regulates extracellular fluid volume by tightly controlling sodium balance. There are direct and indirect relationships between sodium balance, the extracellular fluid volume and blood pressure.

Sodium is the major ion of the extracellular fluid, and the total amount of exchangeable sodium therefore determines both the extracellular fluid volume and blood pressure. Guyton and colleagues have argued that hypertension cannot be sustained without the active participation of the kidney, because elevated renal perfusion pressure leads to water diuresis, returning blood pressure to normal levels (Guyton, 1991). Increased generation of the adrenal hormone aldosterone is a major mechanism in the preservation of salt and water in response to sodium used. However, in the presence of a sustained large salt intake, body fluid volumes do not increased indefinitely; normally, urinary sodium excretion is increased to match the higher sodium intake, thereby preventing overt and dangerous expansion of the extracellular fluid volume. Modulation of renal sodium excretion is a key process in the maintenance of sodium balance.