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Recent Trends in Hypertension Genetics Research

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1. Introduction

Human genome encompasses several thousands of genes which when fail to function normally lead to a defective phenotype expressed sometimes as a disease or disorder. These diseases or disorders may be simple or complex depending upon the nature and number of genes controlling the phenotypes and also their interaction with several other confounding demographic and environmental factors leading to a mosaic pattern of aetiology. Essential hypertension is one such common complex condition prevalent in most of the world populations and stands as a major risk factor for cardio-, cerebro and renovascular diseases (Kearney et al., 2005) adding to the mortality rate when the patients are not treated promptly and managed with proper surveillance. It also causes enormous financial burden to the patients and also nation's economy. This necessitates the need to establish the causes with possible measures for prevention and cure of the condition especially in view of its high prevalence in several developed and developing countries. Research conducted especially in the past two decades were focused more on understanding the etiological factors including genetic components and their interaction with several other factors with a thrust to look for more appropriate therapeutic measures for essential hypertension (EHT).

Regulation of blood pressure is important to maintain adequate blood flow in the body. By definition, blood pressure is the pressure of the blood flowing through the arteries. It depends on the flow of the blood pumped by the heart and the resistance exerted by the blood vessels against the flow. If the pressure is high, heart is forced to work harder. Based on the aetiology, hypertension is further grouped into two major groups viz., a) **Essential or Primary Hypertension** which is caused due to unknown etiology and has no specific origin but is strongly associated with life style habits representing 90-95 % of the diagnosed cases and b) **Secondary Hypertension** which arises due to preexisting medical conditions such as congestive heart failure, arteriosclerosis and disorders of kidney, liver, adrenal and thyroid glands. It accounts to 5 to 10 % of the hypertensive cases and diagnosed by various clinical laboratory and other tests. Apart from these two types, other types of hypertension are also diagnosed based on their characteristic manifestations (*Table -1*)

White coat Hypertension	-	BP high in doctor’s office
Systolic Hypertension	-	High SBP but normal DBP (age related)
Malignant Hypertension	-	Acute uncontrolled Blood Pressure
Labile Hypertension	-	Variable Blood Pressure
Accelerated Hypertension	-	Severe Blood Pressure of recent origin
Borderline Hypertension	-	Blood Pressure in the grey zone
Pseudo Hypertension	-	Due to rigidity of the artery (seen in elders)
Pulmonary Hypertension	-	High Blood Pressure in pulmonary circulation
Renovascular Hypertension	-	Due to narrowed renal artery
Pre-eclampsia	-	Pregnancy induced
Secondary Hypertension	-	Arising from a identifiable disorder

Table 1. Types of blood pressure disorders.

The rhythmic contraction and relaxation of heart creates pressure which is recorded as Systolic blood pressure (SBP) when the heart contracts and Diastolic Blood Pressure (DBP) when the heart relaxes. BP levels are traditionally expressed in millimetres of mercury (1 mmHg = 133 Pascal) in the mercury column of sphygmomanometer and is usually around 120mmHg systolic and 80mmHg diastolic (120/80mmHg) in a normal individual. As per JNC VII (2003) report, based on SBP and DBP levels, individuals are grouped into four classes as given in *Table-2*. An adult with consistent systolic pressure of 140mmHg or higher and/ or a consistent diastolic pressure of 90mmHg or higher is considered to be hypertensive.

	Systolic BP (mm/ Hg)	Diastolic BP (mm/Hg)
Optimal	<120	and <80
Normal	< 130	and < 85
Prehypertension	130-139	or 85-89
Hypertension		
Stage 1 (mild)	140 - 159	or 90 – 99
Stage 2 (moderate)	160 -179	or 100 – 109
Stage 3 (severe)	≥180	and ≥ 110
Isolated Systolic hypertension	≥140	and <90

Table 2. Classification of blood pressure levels.

2. Factors influencing hypertension

Essential Hypertension is a common complex condition displaying substantial public health problem. As a multifactorial condition it involves action of several genes in conjecture with epidemiologic (environmental and demographic) factors manifesting finally into a defined phenotype Hypertension. Hypertension is found to affect 25-35% of adult and 60-70% of elderly population above the age of 70yrs both among the population of developed and developing countries (Staessen et al, 2003). Variation in the prevalence of EHT depends on

the ethnicity of the population like being higher in American Blacks (32.4%) as compared to Whites (23.3%) and Mexican Americans (22.6%) (Oscar et al., 2000). Among American Blacks the condition occurs with greater severity and is associated with high rate of morbidity and mortality due to stroke, cardiac failure, left ventricular hypertrophy and end stage renal disease. While much is known about demographic and environmental factors that predispose an individual to the development of essential hypertension, the nature of the genetic factors that increase susceptibility to the condition remain virtually unknown.

Studies on the epidemiology of hypertension revealed substantial effect of age, gender, body mass index, smoking, high alcohol intake, insulin resistance and also diet with high intake of salt, and low intake of potassium and calcium (Stanton et al., 1982; Appel et al, 1997, Oscar et al., 2000). Some of these factors like obesity and alcohol consumption are additive and modifiable and thus influence variations in blood pressure and expression of hypertension phenotype. Other factors which are non-modifiable like age, gender, genetic factors etc., do not influence the variations in blood pressure and hence the associated risk levels (Fig - 1). In fact the factors like obesity, cholesterol levels etc., remain in an individual more or less stable over time while blood pressure levels keep changing several times even in a day due to the action of multiple physiological pathways to maintain the appropriate levels. Interaction between the hypertensinogenic factors and susceptibility causing genetic factors that have small effects poses a great challenge in unravelling the links, causes and management of hypertension.

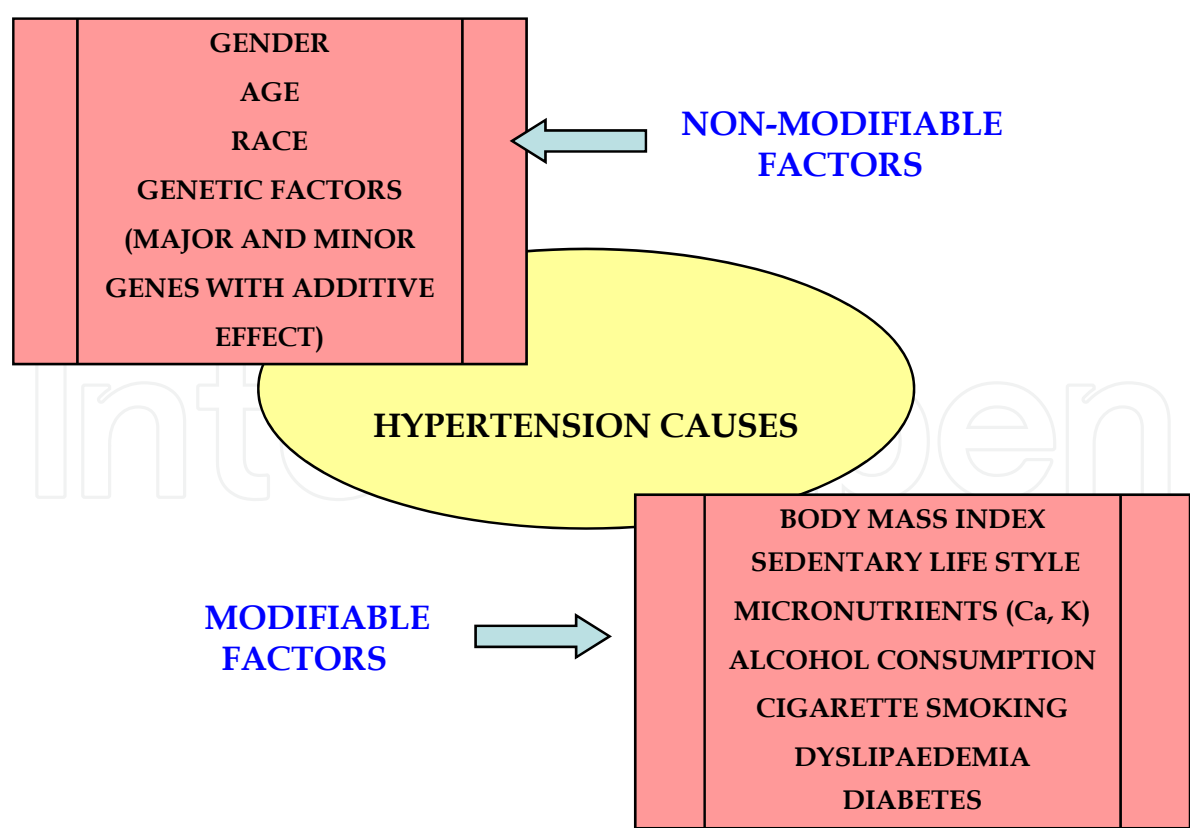


Fig. 1. Modifiable and Non-Modifiable factors influencing onset of essential hypertension.

Age is recognized as a non-modifiable risk factor for high blood pressure with continuous increase in SBP and decrease in DBP levels with ageing throughout ones life. Onset of elevated DBP is usually observed from 35-40yrs due to multiple metabolic, physiologic or genetic reasons while increase in SBP among the aged is observed due to arteriosclerosis or hardening of arteries, decrease in kidney function and physical activity. In many populations men show higher prevalence of hypertension with early onset than women. It has been suggested that estrogen levels have lowering effect on blood pressure in young women but a dramatic increase in the incidence of hypertension is observed in the postmenopausal women. Obesity and overweight also pose a major risk for chronic diseases including hypertension. Obesity induces a high secretion of insulin which brings in many modifications in the body like i) Thickening of the vessels which is responsible for an increase in the rigidity of arteries and in turn the blood pressure, ii) Increase in the cardiac output due to the secretion of increased adrenalin, iii) Induction of reabsorption of water and salt by the kidney which increases the blood volume and consequently the blood pressure iv) Over-sensitiveness to sodium which is known to increase the rigidity of the peripheral arteries.

Body Mass Index (BMI) is a simple index of weight-for-height that is commonly used to classify underweight, overweight and obesity in adults. It is defined as the weight in kilograms divided by the square of the height in meters (kg/m²).

BMI = Mass (Kg) / Height (m)²

BMI of <18.5 - Underweight; 18.5-24.9 - Normal; 25.0 - 29.9 - Overweight; >30 - Obese; >40 - Morbid obesity

Among the addictions, cigarette smoking has been investigated world wide as a risk factor for coronary heart disease (CHD), along with high blood pressure and cholesterol disorders. The nicotine in cigarettes and other tobacco products cause blood vessels to constrict and heart to beat faster, which temporarily raises the blood pressure. Drinking alcohol excessively also is found to increase the frequency of high blood pressure by one and half to two times. Alcohol when present in the blood stream it covers the blood vessels and arterial walls increasing the tension and subsequently the blood pressure. In the recent past emphasis is laid on the use of balanced high blood pressure diet with sparse amounts of saturated and trans-fats and moderate amounts of other fats. Micronutrients are also as important since intake of increased amount of sodium and low levels of potassium and calcium in the diet lead to increases in blood pressure. Increase of potassium in diet helps to balance the amount of sodium in cell fluids and rids cells of excess sodium through kidneys, which filters out extra amount of sodium while inadequate potassium can allow excess sodium to accumulate and thus increases the blood pressure. High levels of intracellular calcium increases vascular smooth muscle tone, peripheral vascular resistance, and responsiveness to the sympathetic and RAS systems that elevate blood pressure. Paradoxically it is low not high calcium intake that stimulates an increase in parathyroid hormone which leads to calcium mobilization from the bone, increased intestinal calcium absorption, decreased renal calcium excretion, increased intracellular calcium concentration with increase in blood pressure. In general, life style factors like lack of exercise and physical activity, sedentary jobs, habit of smoking and consuming alcohol all are also associated with

enhancement in blood pressure and more so when the individuals are genetically predisposed to the condition.

2.2 Genetic factors

2.2.1 Inheritance of hypertension

Inter individual variations in BP levels are known to be inherited with 30% of contribution although the exact genetic factors that contribute to the variations are not clearly established. The concept of genetic basis for hypertension originated 50yrs back itself with the proposal of Platt (1967) who stated that essential hypertension arises due to a single dominant gene. He observed that blood pressure values among the sibs followed a bimodal distribution with two peaks corresponding to systolic blood pressure of 130 mm mercury inherited by the normal gene and those with 160 mm mercury inherited by the gene for hypertension. At the same time Pickering (1967) proposed his view that blood pressure is inherited as a multifactorial condition since the frequency distribution curves (bell shaped) for blood pressure in the relatives of patients were observed to be similar in shape to the distribution observed in the population based sample. The distribution however showed a shift upwards to one extreme or skewed by about the same amount at all ages. Further evidences for the possible involvement of genes for hypertension comes from the population studies exhibiting greater similarity in the measurement of blood pressure within families than between families (Longini et al, 1984). This familial resemblance appears to be present at all levels of blood pressure and hence same genes can be predicted to influence blood pressure over all values- the hypothesis proposed by Pickering. Given an affected first-degree relative, the risk of hypertension is found to be increased 2-5 fold in family members as compared to the population risk. Studies conducted on familial aggregation of hypertension demonstrated correlation in the blood pressure levels between sibs and between parents and children (ranging from 0.14-0.18) and between monozygotic twins (ranging from 0.55-0.58) as opposed to dizygotic twins (ranging from 0.25-0.27) supporting the genetic basis for the condition. This observation is strengthened further by the presence of higher concordance rate among biological sibs as opposed to adopted siblings living in the same household (Mimura, 1973; Biron et al, 1976; Feinleib et al., 1977; Rice et al., 1989; Williams et al, 1991; Oscar et al., 2000). As family members share genes and environment, the correlations regarding blood pressure levels between them may also result due to interaction with shared environment. However, relatively low blood pressure correlations observed between pairs of spouse (0.06-0.08) implicate a substantial influence of genetic factors when family blood pressure correlations are considered. Furthermore, adoption studies indicated higher correlation in the levels of blood pressure between the parents and their biological children than between parents and their adopted children (Biron et al, 1976) emphasising genetic basis for blood pressure.

The strength of a genetic component for a multifactorial condition like hypertension is estimated as heritability, which is defined as the proportion of phenotypic variance due to genetic factors over the total phenotypic variance observed. Heritability encompasses two genetic components causing effects that are a) Additive and b) Dominant effects. Narrow-sense heritability, denoted as h^2 reflects the additive genetic effects and is considered as a measure of predictability of offspring trait values based on parental trait values whereas

broad-sense heritability estimates denoted as H^2 include dominant gene effects which explain part of the heritability resulting due to the effects of major genes. However, these major genes do not generally contribute substantially to the heritability of complex quantitative traits like blood pressure in the general population. Large family-based studies conducted have estimated 20-40% narrow-sense heritabilities for DBP and SBP (North et al, 2003) and around 60% in twin studies (Williams et al, 1991).

Effect of age was also demonstrated on the heritability estimates for blood pressure by the studies on adult twins which was higher than in case of infant twins (Scott et al, 2010). This suggests that blood pressure heritability increases with age. In general heritability of blood pressure attributed to all the genetic factors varies from 25% in pedigree studies to 65% in twin studies.

2.2.2 Genetic determinants

Genes responsible for hypertension in reality may be the ones that are meant to regulate blood pressure levels in an individual. Such genes are not likely to be the causative mutations, but they may be akin to "oncogenes" which are essential to life but may be getting abnormal in function at a given time. So the genes for hypertension are normal but impacted by the confounders raising the blood pressure level leading to clinical expression of the condition. Neel et al. (1998) rightly used the term "Syndromes of impaired genetic homeostasis" to diseases arising due to mismatching of previously adaptive genotypes and modern environmental conditions. In populations without any hypertensinogenic factors blood pressure measurements follow a normal distribution but will be skewed towards right of the curve with narrow base or less variance. With the addition of hypertensinogenic factors like insulin resistance, obesity, high sodium intake, circulating angiotensin levels etc., the distribution may be skewed further to the right, with flatter curve and more variance indicating increase in the frequency of subjects with hypertension who get plotted under the tail end of the distribution (Oscar et al., 2000, Fig-2) The hypertensinogenic factors themselves may possibly have important genetic component and may act as intermediary phenotypes enhancing or decreasing the blood pressure levels like in case of obesity which is observed to be a risk factor for enhancing blood pressure. Severe the obesity greater is the risk observed for developing hypertension. Concerning the molecular basis of essential hypertension, the focused view is that in populations blood pressure has a quasi-unimodal distribution and in a person with higher blood pressure values, higher are the chances of him possessing the disease-type variant alleles of the susceptibility causing gene. Thus greater number of susceptibility genes are likely to be impaired in hypertensives as compared to normotensives (Kato, 2002) (Fig-3).

The advanced molecular biological technologies developed in the recent past helped in identifying as many as 17 independent mutations in genes associated with hypertension (8 genes) or hypotension (9 genes) (Lifton et al., 2001; Table- 3). These monogenic Mendelian forms of hypertension are rare and arise mostly due to the errors in different metabolic pathways. Mechanism underlying these genes does not explain the blood pressure variability observed in populations (Staessen et al., 2003). Hence efforts were focused more on the gene(s) and interacting confounding factors that influence the blood pressure variations leading to the clinical expression of essential hypertension. Several strategies were applied to meet this end which led to the consensus that hypertension is more likely a

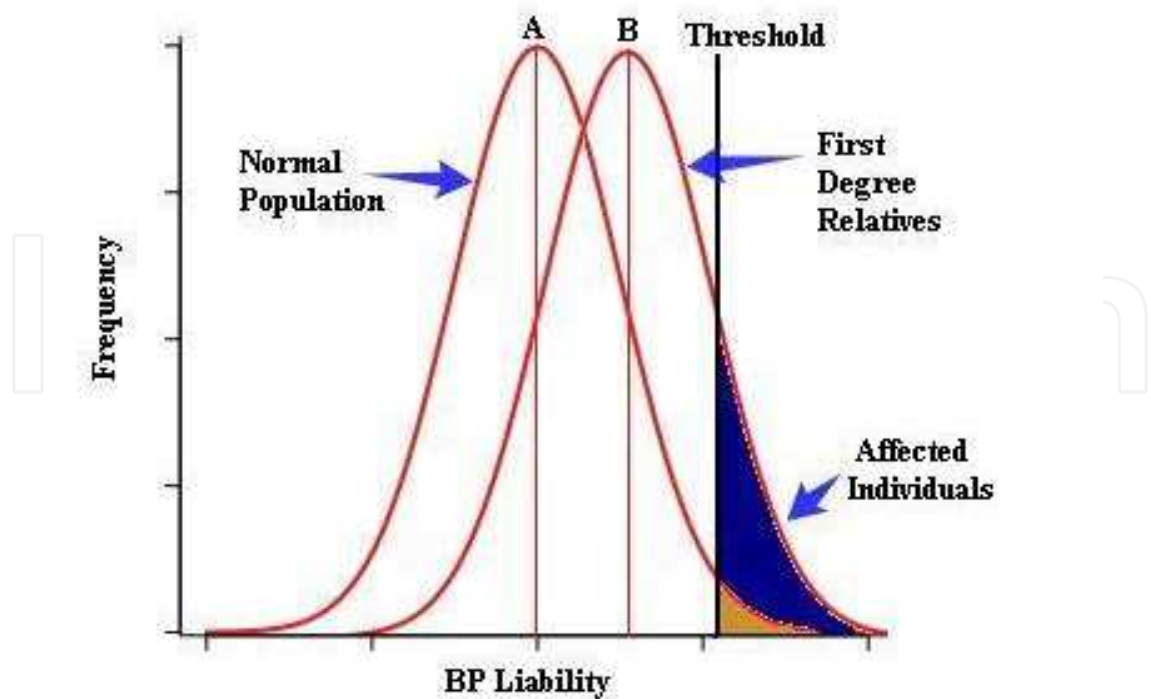


Fig. 2. Hypothetical Curves of liability for BP in general population and relatives of Probands. A- Mean BP levels in population; B-Mean BP levels in relatives of probands.

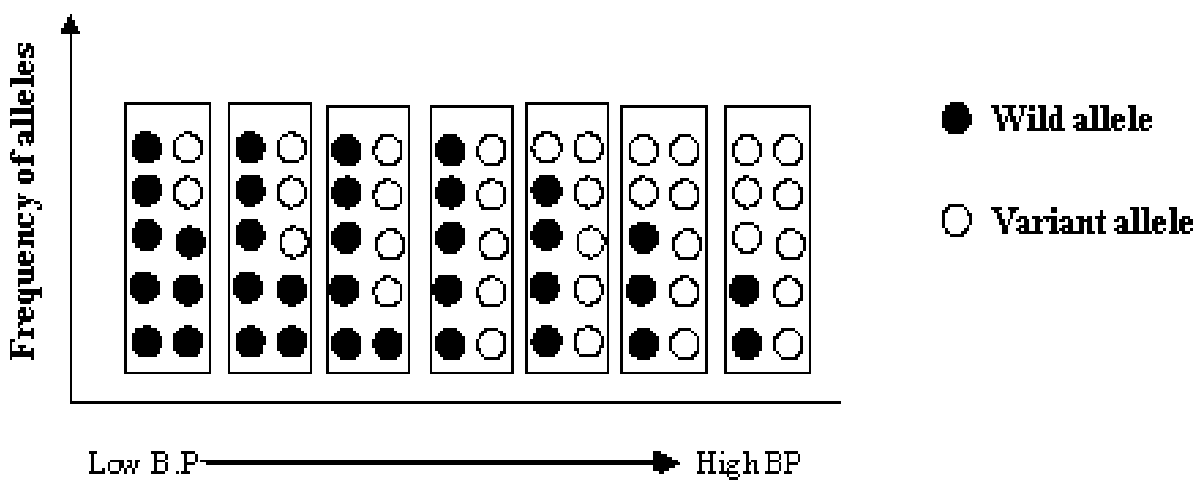


Fig. 3. Linear increase of BP levels with increasing variant alleles.

cumulative burden of many genes with small effects. It is now accepted that hypertension is a multifactorial condition with the interplay of several genes with small effects. The search proved to be tough due to the realities like a) Lack of standardisation and arbitrary dichotomisation of a continuous variable into hypertensive and normotensive groups b) Requirement of very large sample sizes to obtain good statistical power c) Inappropriate selection of controls d) Population admixture and stratification e) Failure to account for confounders like environmental and epigenetic factors which themselves may be associated with independent genetic variants (like obesity, salt and potassium intake etc.), f) Assessment of the effects of environmental factors on genetic variants which develops over

time at different levels in different individuals and finally g) Presence of genetic heterogeneity where inter-individual variations in blood pressure levels are observed with reference to the associated genetic variants and also environmental factors which adds to the reduction in the power of the study considerably. Against all the odds, research on hypertension genetics has made remarkable progress in the last two decades towards better understanding of the condition mainly due to the development of wet lab technologies, biostatistical approaches for data analysis and bioinformatics tools.

Syndrome	Mutations In	Mode of Inheritance
Glucocorticoid remediable aldosteronism (GRA)	Chimerical gene containing promoter area of AS and coding region of 11 β H	Dominant
Apparent mineralocorticoid excess (AME)	Absence of 11 β HSD	Recessive
Liddle syndrome	β or γ subunit of ENaC	Dominant
Pseudohypoaldosteronism type 2	One of the Genes mapped to 1q31-42, 12p13 and 17p11-q21	Dominant
Hypertension induced by pregnancy	Mineralocorticoid receptor	Dominant
Hypertension with brachydactyly	Gene mapped to 12p11.2-12.2	Dominant
Defective aldosterone	Aldosterone Synthase or 21 hydroxylase	Recessive
Dominant Pseudohypoaldosteronism type 1 (PHA1)	Mineralocorticoid receptor	Dominant
Recessive Pseudohypoaldosteronism type 1 (PHA1)	α , β or γ subunit of ENaC	Recessive
Peroxisome proliferators activated receptor gamma (PPAR γ)	PPAR γ	Dominant
Gitelman's syndrome	Thiazide sensitive NaCl cotrans -porter	Recessive
Bartter's syndrome	Ion transporters	Recessive

Table 3. Mendelian forms of blood pressure dysregulation.

3. Methods for identifying genetic determinants

Presently two major approaches viz., a) Association studies b) Linkage analysis are being adopted to identify the genetic components underlying complex diseases including essential hypertension.

3.1 Association studies

This approach is based on comparison of cases with controls to examine whether an allele or genotype of particular **polymorphism** (Table- 4) at candidate gene loci are seen more frequently in disease populations than in a control population. This approach requires careful selection of controls with adequate sample size that has statistical power to validate the results. The controls planned should match with the disease population for parameters like sex, age, ethnicity, socioeconomic status, accurate diagnosis etc. The controls should

also be screened for the presence of hypertension and history of associated conditions like diabetes, thyroid disorders, cardiac disorder, stroke etc., (which may have genes overlapping with genes for hypertension) and should be drawn at random from the same population/ethnic group from where the patient group is selected. The controls drawn from the population are unrelated and thus expected to have random distribution of the marker alleles selected. For age related condition like hypertension which increases in frequency with age, one should be certain that the control subjects are not likely to be the carriers for the hypertension susceptible genes. It is rather difficult to achieve this requirement and one way of reducing the bias in selecting the controls will be to include such of the individuals who are older than the mean age of onset reported for the patient group and also subjects who do not have the family history of the condition. A better alternative choice of controls would be the unaffected normal sibs who are elder to the patients so that they would have crossed the age at expression of the condition in that family at the time of investigation. Further sibs share common environment and hence it would be possible to sort out the genetic component underlying the condition from the environmental factors. The careful selection of the controls can avoid the spurious conclusion on the associations found between the disease and the polymorphic loci.

Type of Marker	No. of Loci	Features
Blood Group antigens	>25	Genotype cannot always be inferred from the phenotypes. Difficult for physical localization
Serum Proteins	30	Often limited polymorphism. Difficult for physical localization
Leaucocyte antigens (HLA)	1	HLA system with A, B, C, D and DR loci each harboring hundreds of alleles thus resulting extensive polymorphism. Is highly informative
Restriction Fragment Length Polymorphisms (RFLPs)	>10 ⁵	Two allele markers, maximum heterozygosity 0.5, genotyped using Southern blotting and PCR techniques. Easy for physical localization
Variable number of tandemn repeats (VNTRs)	>10 ⁴	Many alleles and highly informative. Easy for physical localization. Tend to cluster near the ends of chromosomes
Microsatellites	>10 ⁵	Many alleles and highly informative.Easy for physical localization. Distributed throughout the genome
Single Nucleotide Polymorp-hisms (SNPs)	>4x10 ⁵	Less informative than microsatellites. Can be genotyped on a very large scale using automated equipment

Table 4. Polymorphic genetic markers used in genetic analysis and identification of disease genes.

Association studies rely on the phenomenon of **linkage disequilibrium (LD)** and are conducted to evaluate the risk for developing the disease by the individuals carrying a specific genotype or allele of polymorphic candidate loci. These loci when co-dominant have three genotypes (AA, Aa and aa) determined by the combination of two alleles (A and a). In normal population the distribution of genotypes and the alleles are expected to be in "**Hardy-Weinberg equilibrium**" (HWE) and there may be deviations from the equilibrium when events of migration, genetic drift, selection etc., operate in a given population. Deviations in genotypic and allele frequencies of a marker gene from HWE in a patient population indicate possible association of the disease susceptible gene with the marker allele that may cause risk to the carrier individual of that allele for developing the condition.

Linkage disequilibrium (LD) results when the frequency of one or more gametic combinations of alleles at two loci A and B does not coincide with the combined individual frequencies of the two alleles i.e. $P_{A1B1} \neq p_{A1}p_{B1}$.

In this context the term linkage does not refer exclusively to loci on the same chromosome, but it also includes loci on other chromosomes. Linkage disequilibrium (D) is measured as the product of frequency of gametes with the same type of alleles minus the product of the frequencies of gametes with different types of alleles

$$D = P_{A1B1} P_{A2B2} - P_{A1B2} P_{A2B1}$$

When $D = +0.25$, only gametes with same type of alleles $A1B1$ and $A2B2$ occur in a population. When $D = 0$, the two loci are considered to be in genetic equilibrium

Lower the frequency of recombination between the loci, longer the time it takes for the genes to reach equilibrium. After several generations, the two genes do not reach equilibrium because of absence of crossing over due to absolute linkage between them.

The significance of associations is judged by the values of "**Odds Ratios**" (ORs) computed by comparing patient and control groups for the frequencies of marker alleles/genotypes as a ratio. The ORs obtained for any allele/genotype is compared against a reference value of 1.0, the ratio obtained when the distribution of genotypes of a polymorphism remains more or less similar in both patient and control groups. OR's can deviate significantly from 1.0 and when the OR is significantly >1.0 , then the locus tested is considered to be in positive association with the disease, causing risk to the individuals carrying the particular genotype/allele; when it is significantly <1.0 , it is interpreted that the marker allele is negatively associated and offers protection to the carrier individual against the disease. The ORs are computed for alleles (A vs. a) or for one genotype against any of the other two genotypes (AA vs. Aa; AA vs. aa and Aa vs. aa) and also for one genotype against the pooled frequencies of other two genotypes (AA vs. Aa + aa; Aa vs. AA + aa and aa vs. AA + Aa). These values are also represented as those obtained under "**Dominant Model**" [(which specifically tests the association of having at least one minor allele say 'a' (i.e. in Aa or aa individuals) versus not having it at all (AA individuals))] and "**Recessive Model**" [(which specifically tests the association of having the minor allele "a" in homozygous state (aa) versus having at least one major allele A (Aa or AA individuals)].

Odds Ratios are also computed to predict risk of alleles of individual markers and/or multiple markers. When multiple marker alleles are used to test associations, Bonferroni

corrections is applied to validate the significance of risk obtained for the individual markers studied. In addition, ORs can also be computed for **haplotypes/haploblocks** formed by a set of alleles of marker genes on the same chromosome along with the disease gene. Haplotype associations provide information on the combinations of certain alleles present on the same chromosome as the disease gene. Associations of the disease gene found with certain haplotypes may enhance or circumvent the risk estimates obtained for single marker allele.

The association detected can be positive or negative and the criteria required for high quality positive and negative case control studies are described by Sharma and Jeunemaitre (2000). The criteria suggested for Positive case-control study are - large sample size; well matched controls; accurate definition of the phenotype; a priori estimation of the power of the study; small p-values adjusted to the number of SNPs tested; biological plausibility and functional significance; independent replication in several populations; confirmation in family based studies; high ORs/attributable risk and for a Negative case-control study these criteria are - large sample size; well matched controls; accurate definition of the phenotype; biological plausibility and functional significance; a priori estimation of the power of the study; testing at least three polymorphisms at the same locus; independent replication in at least one other populations; haplotype analysis.

The positive associations of the marker alleles tested with the disease may result due to a) Functional relationship between the marker allele and the disease susceptibility genes b) Pleiotropic effect of the disease causing genes c) Epistatic (Non-allelic) interaction between marker alleles and disease causing genes d) Linkage disequilibrium between marker loci and disease causing loci e) Effect of natural selection. Absence of association may indicate no role of the markers studied in the causation of the disease. The finding could be a false negative resulting obviously due to lack of power and does not refute the hypothesis of association. It should be noted that there is strong inverse relationship between the frequency of a given allele in a population and the number of probands needed to test its contribution to the phenotype particularly with those with low heritability expected like in hypertension (Sharma and Jeunemaitre, 2000). Contribution of rare alleles cannot be ruled out because of lack of power. By increasing the sample size the contribution of rare alleles can be brought to light. Negative results found in a population/ethnic group need not replicate in other populations/ ethnic groups since in different populations different genetic variants may either cause risk or protection to the condition. It is possible that in populations there may be more number of alleles causing susceptibility and less number of alleles counteracting the onset of hypertension. The analysis of intermediate demographic, biological or clinical phenotypes associated with hypertension also may help to subdivide the patient population into homogeneous groups and help in further analysis to achieve more precise results.

Association studies in general are widely pursued because i) The study is easy to conduct based on cases and controls since there is feasibility of collecting large number of samples from unrelated individuals without much difficulty, ii) It does not require any assumption about inheritance pattern, iii) Statistical power of the study is relatively large with the requirement of 1,000 samples to detect the relative risk of 1.5 for a given genotype iv) Candidate genes can be selected for conducting the study based on the physiology and biochemistry related to the disease process v) Small chromosomal region ranging between 3kb - 50kb in size is enough to detect linkage between the putative gene for the condition

and marker genes vi) About 100,000 SNPs are enough to conduct genome wide association scans (GWAS). The only disadvantage in the study is the possibility of obtaining false positives because of population stratification and heterogeneity of the condition. Population stratification can be resolved by studying additional polymorphic loci located on different chromosomal regions and verifying for differences in the allele frequencies in the population. Unaffected family members can be used as controls to avoid the contribution of associated confounding factors. Other limitations of an association study are, it cannot detect genetic linkage of loosely linked loci. Since the approach can detect linkage only when the polymorphism and the disease susceptible gene loci are in linkage disequilibrium, the study can be conducted only by using candidate genes rather than the anonymous DNA markers. It should be remembered that lack of linkage disequilibrium between the loci studied does not necessarily imply absence of linkage. When the marker and disease causing mutation loci are located several million base pairs away, the linkage can still be detected using more powerful techniques to analyse the data.

3.2 Linkage analysis

Linkage is a phenomenon that deals with the tendency of any two traits (phenotypes, marker alleles etc.) to co-segregate among family members of extended pedigrees because the loci that harbor these genes are physically located on the same chromosome with defined distance. The strength of the linkage depends on the distance between the two loci which is represented as Centi Morgan (cM). cM is a unit that indicates the probability of recombination, consequence to the crossing over occurring between the two loci present on the same chromosome and is expressed as percentage. Greater the distance between the two loci, higher will be the frequency of crossing over leading to recombination occurring between the loci being tested. The analysis of linkage is carried out as **a) Two point analysis** where the distance between two loci i.e. disease causing locus and marker locus are assessed and **b) Multipoint analysis** where several loci presumably including the disease causing locus are tested for the distance between them. Multipoint linkage analysis helps to resolve the order of location of the candidate loci and the position of the disease causing locus in relation to them. It also identifies the candidate loci flanking the disease gene locus. Linkage between any two loci is computed as lod score based on which distance between any two loci can be determined that is expressed in terms of Centi Morgans or cM. Lod Score analysis can be **i) parametric or ii) non-parametric**. For parametric estimations a precise genetic model or inheritance pattern for the condition under study should be known along with the gene frequencies and penetrance of the genotype of the markers studied. With valid model it stands as a powerful method to scan and localize Mendelian disease genes within 20-Mb segment of the chromosome. In contrast, nonparametric method is applicable for conditions for which precise inheritance pattern can not be assigned like in hypertension. It is also referred as “model free” method and depends on identifying the alleles or chromosomal segments that are shared by affected individuals in a given family or population, resulting in resemblance/concordance for the two traits being studied.

3.2.1 Parametric method

Parametric method is applied for localizing/mapping genes for the condition with definite model of inheritance. For the mapping of gene(s) for an autosomal dominant condition,

conventional method of Linkage analysis is carried out based on the estimation of likelihood of the genetic marker loci (Table -4) which are polymorphic with high heterozygosity that co-segregate along with disease among the family members through generations. It is based on the estimation of Lod score (L) that is defined as the likelihood of obtaining a family/pedigree with the segregation of marker and disease loci together in the presence of certain amount of recombination between them (depicted as θ or x or r) and which varies from 0.0-0.5) compared to the likelihood of obtaining the same family/pedigree when there is no recombination between the marker and disease loci (i.e. θ or x or $r = 0.5$) i.e. when the recombination frequency between the two loci is maximum which means that there is absence of linkage

Maximum likelihood estimate (mle) of recombination fraction (referred as θ or x or r) is used for determining linkage between two loci on a chromosome based on the relative probability (P_R) of having obtained the family. The P_R is determined by calculating the probability of having obtained the various combinations of the particular trait under consideration on the assumption of there being no measurable linkage (H_0 = No linkage; $\theta = 0.5$) and comparing this with the probabilities based on a range of recombination fractions θ varying from 0.00 to 0.05, i.e.

$$P_R = \frac{P(\text{FAMILY, GIVEN } \theta = 0.0 \text{ to } 0.5)}{P(\text{FAMILY, GIVEN } \theta = 0.0 \text{ to } 0.5)}$$

P_R is expressed as its logarithm. The \log_{10} of P_R is called as log of the odds or lod score. Lod score value of ≥ 3.0 indicates that the two loci tested are linked, value of 2.0 as evidence of strong linkage, value of 1.0 as evidence for tentative linkage and that of -2.0 absence of linkage. The Lod score of 2.0 that is suggestive of linkage can be further evaluated by analyzing more candidate/marker loci and by screening additional members of the family.

Large extended pedigrees with valid power are required for linkage estimations and gene mapping of an autosomal dominant condition. Extent of penetrance and population frequency of the gene in question and information on phase of linkage are required while using this method.

For an autosomal recessive condition where the parents of the affected individual are invariably blood relatives and heterozygous, different approach is adopted. It is ascertained whether both the alleles in the homozygous affected inbred individual are demonstrably transmitted representing copies of an allele present in a common recent ancestor. Such alleles in the homozygotes are referred as **"Identical by descent" or as IBD**. When the alleles carried by an individual are identical, but are not demonstrably inherited from the common ancestor implying that they have two different origins, then they are referred as **"Identical by state" or as IBS** (Fig- 4). Hence IBS analysis is based on population frequencies and not on Mendelian probabilities. The inbred individual is expected to be homozygous for the segment of the chromosome with some loci flanking to the disease causing locus. By examining the segregation of such flanking loci (haplotypes) from the phenotypically normal but heterozygous parents to the affected offspring, one can estimate whether the disease causing locus and the candidate loci are linked based on the extent of recombination occurring between the loci studied. For linkage analysis of an autosomal

recessive condition less than 10 pedigrees with the affected offspring and parents are considered adequate. There is no need to have information on the extended pedigrees. This method is useful for identifying genes for rare autosomal recessive conditions where the parents are invariably blood relatives/cousins and hence have higher chance of inheriting same mutant allele from the common ancestor. Also the method is suitable to study the small families with affected children but phenotypically normal parents who could be the founder members in small communities. For very rare alleles, two independent origins of alleles in the homozygotes are unlikely and hence IBS will become similar to IBD. This is unlikely with common alleles where there is possibility of having identical genes in the homozygote individual that are not IBD in origin. For IBD analysis multiple loci with multiple alleles logically will yield better results. Both IBD and IBS methods can be used to estimate linkage by examining the shared chromosomal segments or alleles for nuclear families (sib-pair studies for concordance/resemblance for the two loci) or in the whole population descending from FOUNDER population. IBD method of analysis is more powerful but the study has to be conducted using large number of affected relatives specially the parents.

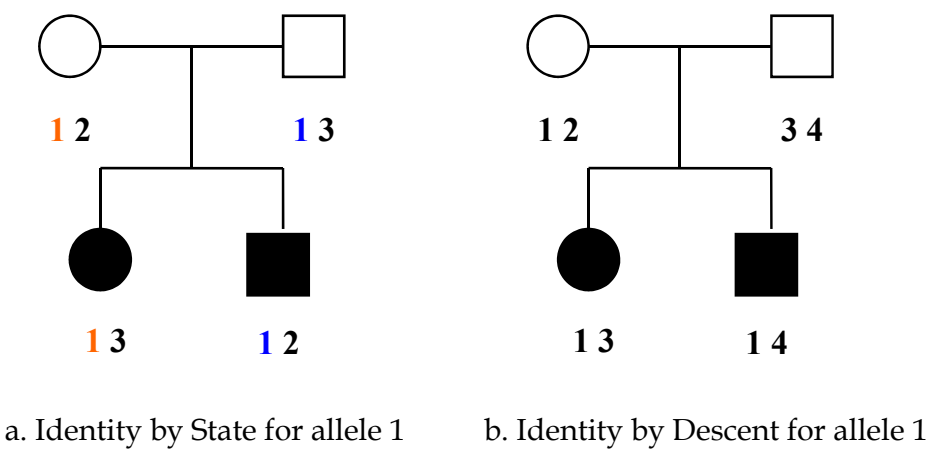


Fig. 4. Pedigrees showing transmission of alleles that are identical by state (IBS) and identical by descent (IBD).

3.2.2 Non-parametric or model free method

3.2.2.1 Linkage disequilibrium (LD) mapping

Linkage disequilibrium may result due to a mutation in individual/individuals in the world population at some point in human history. These mutations would have segregated in the following generations along with the neighboring loci on the affected chromosome. In successive generations the recombination events scramble the alleles of the loci surrounding the disease causing gene. After several generations, the disease genes and the loci that are closely linked with it from the original chromosome remain together with no chances of further recombination between them. As a result the frequency of the two linked genes exceeds or will be in disequilibrium with that predicted by multiplying together their individual frequencies. This phenomenon referred as linkage

disequilibrium (LD) is defined as non-random association of alleles at two or more loci present on the same or different chromosomes. The region of the chromosome with disease and linked neighbouring loci form a part of the unique haplotype on the ancestral chromosomal fragment and such segments help in mapping the genes more precisely for monogenic or multifactorial conditions. The distance between the loci mapped through LD estimations is often less than 1-2 cM, lower limit that can be resolved by conventional genetic linkage studies. Now several free soft wares like Haploview, PLINK, Gene hunter etc., are available to evaluate the level of LD, haplotype associations with disease causing genes, LD mapping, etc.,

LD mapping can be extended to nuclear families by performing “**Transmission Disequilibrium Test**” (TDT).

Transmission disequilibrium Test (TDT), is a family-based study to test whether a marker is associated with the disease. Families with parents and one or more children are used for the test. It is irrelevant if either parent is affected. or not. For this test parents who are heterozygotes for the marker allele are selected and number of offspring with the allele transmitted are compared with those without the transmission. The test is not affected by opulation stratification. An extended TDT test, ETDT is developed to handle data from multiallelic markers like microsatellites. The test can be applied when only one parent is available but the results may be biased. When both the parents are not available, sib-TDT can be applied. The test involves genotyping of the probands and their parents and selection of heterozygous parents (they may or may not be affected). If “a” is the frequency with which a heterozygous parent transmits the marker X1 to the affected offspring and “b” is the frequency with which the other allele X2 is transmitted, then TDT is tested by the statistic which follows chi-square distribution with 1 degree of freedom

$$\chi^2 = (a-b)^2 / (a+b)$$

3.2.3 Sib pair method

Identifying disease genes for hypertension following conventional Lod Score method is not possible because recombination events cannot be detected and mode of inheritance can not be easily modeled. Further the condition is age dependent and often parents of the patients will not be available for evaluation. Information on the offspring of the patients is also not useful as they would not have reached the mean age of expression of the condition in a given family and also the population from where the patients are drawn. Hence the family studies will have to be horizontal since reliable information about the disease can be obtained only from the sibs of the affected who will also be available for clinical and laboratory investigations.

Sib-pair analysis which is based on concordance or co-occurrence of marker alleles and disease susceptibility gene among pairs of sibs in multiple families is considered to be a more powerful method when compared to association studies as it does not depend on phenomenon of LD as is the case in the latter. It is a robust test for linkage analysis particularly when the inheritance model cannot be predicted. For any given locus parents carry four distinguishable alleles whose transmittance to the offspring can be traced. The sib pairs can be grouped into four categories where pairs may inherit both, one or none (2, 1, 0)

of the alleles from the parents with the probability of 25%, 50% and 25% (expected ratio of 1:2:1, Fig-5).

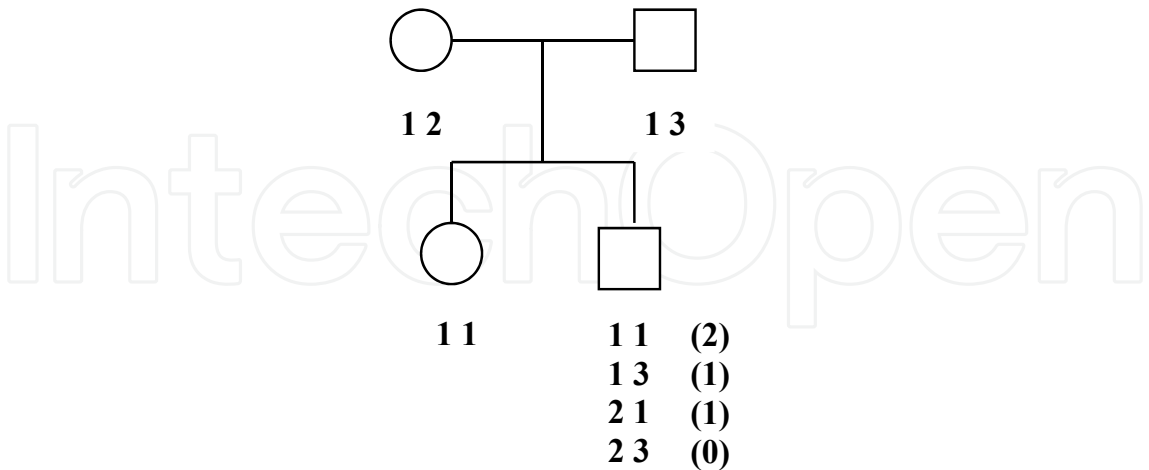


Fig. 5. Pedigree showing allele sharing between the sib pairs. Numbers in parenthesis denote the expected number of alleles shared by the sibs under Mendelian segregation.

If both the sibs in a pair are affected with a genetic disease then they are expected to share segment of the chromosome carrying disease locus. Affected sib pairs (**ASP**) are genotyped for the selected markers, and chromosomal region is searched where sharing by the sibs is above the expected 1:2:1 ratios of sharing 2, 1, and 0 alleles that are IBD. If the sib pairs are tested for IBS, the deviation from expected 1:2:1 ratio should be based on population frequencies. ASP analysis is a preferred method for studying conditions like hypertension since it is easy to identify and collect the sib pairs than the affected families and the analysis is model free. Multipoint analysis rather than the single point analysis is preferred since it offers opportunity to extract IBD information about alleles on the shared chromosomal segment (**haplotypes**) more efficiently. The lod scores computed in ASP analysis is indicated as NPL scores. The chromosomal segment identified by ASP analysis is usually large and not feasible for positional cloning. Yet the method stands as a main tool to search for the susceptibility genes for common non-Mendelian diseases like hypertension in view of its simple approach and robustness.

3.3 Epistatic interaction

Identification of interaction between genes and genes and environmental factors in explaining complexity of human diseases has been challenging. There is increasing evidence that epistasis or gene-gene (non-allelic) interactions play an important role in determining an individual’s risk for developing complex diseases like hypertension (Williams teal, 2000; Tsai et al., 2003). New tools are developed to detect such synergistic interaction between alleles/genes arising due to epistatic phenomenon and modification of phenotypes like gradation in severity etc., due to additive effects or simple correlations between them. One such tool that is being used in interpreting the results for conditions like hypertension is “**multifactor dimensionality reduction (MDR) analysis**” - a nonparametric, model-free test alternative to logistic regression for detecting and characterizing nonlinear interactions among discrete genetic and environmental attributes.

MDR method combines attribute selection, attribute construction, classification, cross-validation and visualization to provide a comprehensive and powerful data mining approach to detect, characterize and interpret nonlinear interactions. The method is effective in detecting multilocus interactions among different polymorphisms of many different genes. It is a data reduction strategy (Ritchie et al, 2001) and in this model multilocus genotypes are pooled into high risk and low risk groups, effectively reducing the dimensionality of the genotype predictors from N dimensions to one dimension. The new one-dimensional multilocus genotype variable is evaluated for its ability to classify and predict disease status using cross-validation and permutation testing. MDR is advantageous program as it identifies evidence for high-order gene-gene interactions in the absence of any statistically significant independent main effects in simulated data (Ritchie et al, 2003).

4. Strategies

Several strategies are being used while searching for the genes causing susceptibility to essential hypertension such as i) Candidate gene approach, ii) Use of intermediary phenotypes, iii) Genome wide Association scans (GWAS) ,.iv) Animal models. Combination of two or more of these methods is also used.

4.1 Candidate gene approach

Candidate genes are defined as the genes that might be responsible for the onset of an inherited disorder. They are identified without reference to their chromosomal location. First a candidate chromosomal region is identified by linkage studies etc., and then the candidate genes from within the chromosomal region by verifying from the list of human genes available on the websites (NCBI; HuGe Literature Finder). From among the possible candidate genes thus identified, the one that is likely to be involved in biochemical, cellular or physiological functions contributing to the disease process are screened for mutation(s) that are likely to affect the structure, expression or function of the protein(s) associated with the disease development. The potential role of the candidate genes can be examined by using the methodologies adopted for linkage and association studies.

Now more than 350 candidate genes have been implicated for hypertension that involves several different biochemical pathways (HuGe Literature Finder). Candidate genes for hypertension are selected logically based on already established effect on cardiovascular and renal function and on the basis of known pathophysiology of hypertension. Genes for monogenic forms of hypertension identified by molecular pathology for cases mentioned in Table-3 also act as candidates to study essential hypertension. A variety of candidate genes have been identified for essential hypertension that are related to the RAAS pathway, sodium epithelial channel, adrenergic function, renal kallikrein-kinin system, alpha adducin, endothelial dysfunction, lipoprotein metabolism, hormone receptors, growth factors and many more (Table - 5).

Several studies from different ethnic groups demonstrated association of the potent candidate genes with essential hypertension that showed positive and negative results. These studies could not be replicated in different populations and the reason for the inconsistency in the results is mainly attributed to the heterogeneity in the samples studied leading to type 1 (false positive) and type 2 (false negative) errors. Lack of statistical power due to small samples screened and use of improper controls are among the other reasons.

Genes	Chromosomal Location/Exons/Introns	OMIM ID	SNP Associated with EHT	Population	Reference
RENIN ANGIOTENSIN ALDOSTERONE SYSTEM					
Angiotensinogen (AGT)	1q42-q43; 5; 4	106150	M235T	Caucasian Japanese African Caribbean Taiwanese Chinese Caucasian Canadian African-Americans Taiwanese Chinese Taiwanese --	Jeunemaitre et al, 1992 Caulfield et al, 1994 Kamitani et al, 1994 Caulfield et al, 1995 Chiang et al, 1997 Fang et al., 2010 Jeunemaitre et al (1992) Hegele et al (1994) Sudhir et al., 2002; Kumar et al., 2005 Wu et al., 2003; 2004; Liu et al., 2004 Tsai et al (2003) Jeunemaitre et al (1997), Wang et al (2002) Li et al (2004)
Renin (REN)	1q32; 10; 9	179820	18-83>A -4021C>T & -3212C>T -5312C>T	Japanese UAE Gulf US population of African origin Whites	Okura et al, 1993 Frossard et al, 1998 Frossard et al, 1999 Niamh et al, 2007
Angiotensin Converting Enzyme (ACE)	7q23.3; 26; 25	106180	Insertion/Deletion Polymorphism in intron 16	African Americans Chinese Japanese Malaysians South Indian	Duru et al, 1994 Jeng et al, 1997 ; Chiang et al , 1996 Nakno et al, 1998 Vasudhevan et al, 2008 Bhavani et al, 2004
Angiotensin II AT1 Receptor (AGTRI)	3q21-25; 5; 4	106165	1166A>C	Whites	Bonnardeux et al , 1994

Table 5.

Aldosterone Synthase (CYP11B2)	8q21-22; 9; 8	124080	-344C>T	French Scottish Japanese South Indian Tamil	Brand et al, 1998 Davies et al, 1999 Matsubara et al, 2004 Rajan et al, 2010
SYMPATHETIC NERVOUS SYSTEM					
β2 Adrenergic Receptor (ADRA2B)	5q31-32; 1; 0	109690	nine bp insertion/ deletion (I/D) in third intracellular loop		Lockette et al, 1995 Wowern et al, 2004
ENDOTHELIAL DYFUNCTION					
Endothelial Nitric Oxide Synthase (NOS3)	7q36; 27; 26	163729	CA repeat in intron 13 (33 repeat allele) 786T>C in 5' flanking region 27 bp VNTR in intron 4	Japanese Canadian South Indians Chinese South Indians	Nakayama et al, 1999 Hyndmann et al, 2002 Sushma et al, 2011 Wang et al, 2010 Sushma et al, 2009
ION TRANSPORT					
β 3 subunits of G-protein (GNβ3)	12p13; 11; 10	139130	825C>T	Caucasian	Schunkert et al 1998; Hengstenberg et al 2001
RENAL KALLIKREIN KININ SYSTEM					
Kallikrein (KLK1)	19q13.3; 5; 4	147910	-58C>T poly-guanine length polymorphism	African Americans Chinese Han	Gainer et al, 2000 Hua et al, 2005
LIPID METABOLISM					
Lipoprotein lipase (LPL)	8p22; 10; 9	609708	Intron 8 polymorphism S447X	Chinese Han	Xin et al, 2005 Salah et al,2009
HORMONE RECEPTORS					
Prostacyclin Synthase (PNMT1)	17q; 10; 9	171190	9bp(CCGCCAGCC) repeat in promoter	Japanese	Naoharu et al, 1999

Table 5. (contd.) Some of the candidate genes reported with positive association for essential hypertension.

Selection of wrong candidate genes, location of the causative genes upstream or downstream from the candidate genes studied and discovery of new pathways for which candidates are not yet identified also contribute to the lack of success in some of the studies conducted.

4.1.1 Candidate genes associated with essential hypertension

Table -5 depicts some of the positive associations reported between essential hypertension and polymorphic candidate genes related to various pathways.

RAAS Pathway: The RAAS is an important regulator of cardiovascular function and blood pressure (Soldner et al, 1996; Ferrario et al, 2006). It consists mainly of 4 genes including renin (*REN*) that converts angiotensinogen (*AGT*) to angiotensin I which is metabolized by angiotensin-converting enzyme (*ACE*) to form angiotensin II, which can act on angiotensin II type 1 receptors (*AGTR1*) to mediate blood pressure elevations by mechanisms including direct effects on vascular tone and indirect effects via alterations of renal function. Thus *REN*, *AGT*, *ACE* and *AGTR1* act synergistically on the phenotype of blood pressure. Each of the corresponding genes has several polymorphisms that can be associated with altered expression or function of the corresponding gene product. While each of these polymorphisms may potentially affect the regulation of blood pressure, most studies have focused on only few polymorphisms in each of these genes i.e., M235T, -217G>A, T174M, -20A>C of the *AGT* gene, an insertion deletion polymorphism involving 287bp in intron 16 of the *ACE* gene and the 1166A>G in the 3' untranslated part of the *AGTR1* gene.

The 235T allele of the *AGT* gene is associated with a step wise increasing level of circulating angiotensinogen (gene dose response; Sethi et al, 2003). The positive correlation between polymorphism M235T and *AGT* plasma concentration has been observed in different populations (Mettimano et al, 2001; Agachan et al, 2003; Yan et al, 2006). Allele *AGT**235T shows linkage disequilibrium with a variant in the *AGT*-gene promoting area - a replacement of adenine by guanine in nucleotide 6 (-6A>G). It has been suggested that such mutation -6A>G interferes in the interaction of transcriptor factors with *AGT* promoter, thus influencing the gene transcription baseline rate. An increase in *AGT* gene expression may increase the production of angiotensin II by RAAS, thus resulting in the expansion of blood volume, which in turn would increase blood pressure. *AGT* gene is primarily expressed in the liver and adipose tissue, and C/EBPs family transcription factors play a crucial role in regulating expression of a number of genes in these tissues. A study conducted by Sudhir et al (2002) showed that recombinant glucocorticoid receptor (GR) and C/EBP family transcription factors bind strongly to nucleoside A compared with nucleoside G at -217 position of *AGT* promoter region resulting in increased transcriptional activity of -217A allele which may be involved in essential hypertension. Further, -20A>C located between the TATA box and the transcription initiation site is thought to modulate gene expression in a gender specific manner (Zhao et al, 1999). Tomoaki et al (1997) demonstrated that plasma angiotensinogen levels increase linearly with the number of -20C allele and that the subjects with the CC genotype are associated with the highest plasma levels of *AGT*. In in-vitro studies by Zhao et al (1999) the A-20C polymorphism has been found to influence basal transcription of angiotensinogen as well as stimulate transcription in response to receptor binding.

ACE insertion/deletion polymorphism was characterized in 1990 and studies have suggested that this polymorphism interferes in *ACE* serum concentration. DD Genotype individuals would have the highest *ACE* serum concentrations (Sayed et al, 2004; Mondry et al, 2005) and it is estimated that allele D would contribute with approximately half the variation of *ACE* plasma levels (O'Donnell et al, 1998). Significant gender differences were also reported in *ACE* I/D polymorphism. Compared with the II genotype, DD homozygosity was associated with higher risk of hypertension in studies conducted on women (Gu et al, 1994; Kiema et al, 1996; Sipahi et al, 2006) whereas two other population studies (O'Donnell et al, 1998, Higaki et al, 2000) found that the D allele was significantly associated with hypertension in men but not in women.

1166A>C at position 1166 in the 3' untranslated (UTR) region of the *AGTR1* gene was identified and was significantly shown to be associated with hypertension by Bonnardeux et al (1994). However it is not clear whether this polymorphism alters mRNA polyadenylation or destabilization signal. It is possible that this polymorphism may be in LD with a functional variant located elsewhere in the *AGTR1* gene or within a nearby gene that could explain the observed associations of this variant with cardio vascular phenotypes (Abdollahi et al, 2005). Therefore, this suppression may lead to the loss of cellular capabilities which protect against an excessive angiotensin II action (Grzegorz et al, 2002).

The renin-angiotensin system thus plays an important role in the inter-related hormonal mechanisms that regulate blood pressure and electrolyte/blood volume homeostasis. The RAS is activated when there is loss of blood volume or drop in blood pressure (such as in a hemorrhage) or low concentration of sodium in plasma, whereas factors that increase these parameters tend to suppress its function.

Sympathetic Nervous System: The sympathetic nervous system has also been implicated in the cardiovascular physiology and hypertension. The sympathetic nervous system primarily regulates the cardiovascular physiology by the release of catecholamines and activation of adrenergic receptors (AR). The human α_{2B} -adrenoceptor is encoded by the *ADRA2B* gene located on chromosome 2q12 (Regan et al. 1988). A common variant form of the this gene which encodes a receptor protein with a insertion/deletion (I/D) of three glutamate residues located in the third intracellular loop of the receptor has been associated with EHT (Lockette et al.1995; Wowern et al. 2004).

Endothelial Dysfunction: Of the substances exerting their effect on vascular endothelium, Endothelial Nitric Oxide Synthase (*NOS3*) and Endothelin 1 (*END-1*) play an important role in regulating vascular tone. *NOS3* gene synthesizes Nitric Oxide (NO), which is a potent vasodilator and the *END-1* gene product acts as a strong vasoconstrictor called endothelin. Endothelin exerts its physiological functions via its receptors on the vascular smooth muscle cells (VSMC), by activating cascade of intracellular molecules that increase the calcium ion concentration, resulting in vasoconstriction. However, NO lowers these calcium ions to basal level, to relax the constricted VSMC, resulting in vasodilation. Thus, the delicate balance between the NO and endothelin is required for smooth functioning of vascular tone. Generation of reactive oxygen species (ROS), from various metabolic pathways, scavenge the NO, reducing the NO bioavailability leading to endothelial dysfunction. Polymorphisms in *NOS3* and *END-1* genes have been associated with hypertension in some populations.

Lipid Metabolism: Essential Hypertension has been frequently associated with serum lipid abnormalities. This suggests that there may be common underlying genetic determinants between blood pressure regulation and lipid metabolism. Membrane ion transport, which has been related to hypertension can be altered by lipid abnormalities and could have some involvement in the mechanisms that link high triglyceride concentrations and hypertension. *LPL* is thought to function as a "gate keeper" for fatty acid (FA) uptake into organs (Greenwood, 1985); however, patients with *LPL* deficiency have no obvious defect in adipose tissue. To date, only a few studies have explored the relation between the genetic variants near or in the *LPL* gene and blood pressure variation. Positive linkage or association has been described in some studies, which indicate that the *LPL* gene may be an important candidate for affecting the risk of Essential Hypertension or BP variation (Sass et al 2000; Pan et al 2000; Wu et al 1996; Chu et al, 2001).

Ion Transporters: G-Proteins are expressed in all cells of the human body and their main role is to translate signals from the cell surface into a cellular response. Polymorphisms in gene coding for G-protein subunits are expected to have a significant effect on the cardiovascular system. Hence, studying the genetic polymorphisms of the components of this system might give a new insight into the genetics of hypertension and may suggest novel downstream targets for therapy.

In addition to the above, a number of other candidate genes have been investigated as contributing to hypertension in case control studies including those involved in renal kallikrein-kinin system, growth factors (TGF- β), cytoskeletal proteins (alpha adducin), hormone receptors (*PNMT1*) etc.,

4.2 Intermediate phenotypes

Difficulties were observed in conducting linkage studies for hypertension using multigeneration families mainly due to the heterogeneity factor, lack of clear cut inheritance model and substantial environmental influence. It is now understood that "intermediate phenotypes" may help resolving this problem to certain extent (Dudley et al, 1992). It is based on the view that the individual genetic determinants having specific biochemical, physiological, demographic phenotype may contribute to the increase in blood pressure levels leading to hypertensive state. These phenotypes determined by single genetic loci, segregate in families following Mendelian inheritance. Such families showing intermediate phenotypes can be identified and the locus causing the phenotype may be detected. Since intermediate phenotypes are directly determined by the action of a particular gene, the influence of environment will also be less as compared to complex conditions like hypertension. Use of such phenotypes with greater heritability and early penetrance, have several advantages over the use of blood pressure phenotype as such. It helps, in identifying homogeneous group among the heterogeneous patient population like in hypertension. It also enables selection of large samples because even younger patients who are still normotensive can be identified with genetic risk for hypertension in the preclinical stage itself and this information will be useful for genetic analyses and at arriving better conclusions. In addition, it enables mapping of quantitative traits loci (QTL) in sibling pairs simultaneously testing for intermediate phenotypes. This approach is expanded to accommodate nuclear families of any size and also Transmission Disequilibrium Test (TDT; Timberlake et al., 2001). Ideal intermediate phenotype is

suggested by these authors to have the features like - an association with essential hypertension; high heritability; high penetrance; early onset among offspring of affected parents; a bimodal distribution; causal role in the pathogenesis of elevated blood pressure; should suggest the candidate genes for testing hypertension. Further the intermediate phenotypes should be easier to genotype by noninvasive methods to test the relatives of the probands since invasive tests might deter the relatives from participating in the study.

One of the simple and measurable intermediate phenotype that enhances the blood pressure to the level of clinical expression of hypertension is obesity/overweight. Obesity is considered as an hypertensinogenic factor that causes elevation in blood pressure leading to greater skewness in the normal distribution curve of blood pressure levels (Fig-2). So individuals with obesity, especially morbid obesity can be considered as those at higher risk for hypertension. In other words obesity can be considered as one of the preclinical symptoms for essential hypertension.

Williams et al., (1992) described a phenotype called “non-modulation” characterized by non-modulation of aldosterone secretion and renal blood flow by angiotensin II infusion in a subset of hypertensive patients. i.e. the response is blunted to angiotensin II infusion. This parameter can be used to identify subjects at risk for developing hypertension in the patients at later years. Among other examples of intermediate phenotype useful for identifying candidates for screening for hypertension include M235T polymorphism located in exon 2 of *AGT* gene. The substitution of Methionine by Threonine at 235 position of the gene has been associated with increased levels of angiotensin in circulation leading to vasoconstriction and high blood pressure (Hopkins et al., 1996). Age, gender and interaction between genotypes have been described to contribute to this trait (Williams et al., 1992). In another instance cases with salt resistant hypertension have been associated with increased levels of urinary free cortisol with bimodal distribution and is considered to identify the new subset among hypertensives (Litchfield et al., 1998). Similarly urinary excretion of kallikrein which is an index of renal tissue expression of kallikrein has been associated with salt sensitive hypertension and hence can be used as intermediate phenotype for screening for essential hypertension specially in African-Americans (David et al., 2001). Other promising intermediate phenotypes are related to physiology and biochemistry of autonomic and sympathetic nervous system functions that can identify several subsets of hypertensive patients. In the following years list of intermediate phenotypes is expected to emerge that can be used successfully in linkage and association studies and would help in better understanding of mechanism underlying hypertension apart from resolving heterogeneity related with the condition.

4.3 Genome Wide Scans (GWS)

Since no single gene is solely responsible for the development of hypertension and it is tedious to study all the 350 implicated genes one by one for their causal role, genome wide scans (GWS) are attempted to identify susceptible regions on chromosomes comparing microsatellites/SNP variations that are spaced at regular intervals of 10-30 cM across the genome among large number of patients with appropriate controls and through linkage analysis, mapping and genome wide association studies (GWAS). These regions then can be searched for positional candidate genes and possible mutations in them, to conclude on the mechanism of the disease process and explore better management measures.

Genome wide scanning (GWS) is an indirect strategy in which affected individuals, siblings or family members with more than two affected individuals are genotyped for a number of polymorphic DNA markers (short tandem repeats and simple sequence repeats) covering the entire chromosomal complement/genome. A set of about 300-400 short tandem polymorphic repeats that are spaced for every 10cM is used in most genome scans. This covers approximately 3000 cM of the human genome. Later simple sequence repeats or microsatellites with an assembly of 3000 markers that are spaced with a distance of 1.5 cM were used to obtain better resolution of the genome map. Further resolution was achieved with the discovery of single nucleotide polymorphisms (SNPs) that are distributed for every 300 bases in the genome. They are $\sim 4 \times 10^6$ in number and are easily identified with automated sequencing and other techniques like restriction fragment length polymorphisms (RFLPs). Being more stable and non-mutable, screening for SNPs as markers is now pursued in genome scan attempts to detect linkage by LD mapping between marker loci and quantitative trait locus (QTL) like hypertension. Significance of the results obtained is determined based on the lod scores plotted as a function of the location in cMs along the genome. Generally lod score value of ≥ 3.6 is considered significant indicating presence of linkage while a value of > 2.2 is considered suggestive and the value > 1.5 is considered as interesting. Additional polymorphic markers and inclusion of more number of patient samples and family members can improve the lod scores obtained and reduce the events of false negative results.

In human genome there are regions of high LD occurring as blocks ranging from 10 to > 100 kb separated by regions of little or no disequilibrium. Each block with high LD comprises small number of haplotypes and study of these haplotypes or haploblocks reduces the number of SNP markers to be screened to detect linkage or association with the disease gene with required precision and resolution. SNPs can be selected from International HapMap Project – a global consortium that has the information on all common SNPs recorded from different populations across the world. Through the genome wide association scan (GWAS) approach, SNPs from this project are selected to study the common diseases and traits based on “common disease common variant” (CDCV) hypothesis (Doris, 2002). In GWAS approach no assumptions are made about the location or function of the variant. Now genotyping micro chips with thousands of SNPs are available that offer much better coverage of human genome. Depending on the population/ethnic group studied relevant informative SNPs/tag SNPs from within or outside the genes can be selected and the GWAS can be conducted using $\geq 300,000$ SNPs.

GWAS scan the genome for association signal without selecting for gene regions. Gene-centric approach to GWAS was proposed by Jorgenson and Witte (2006) since genic variants are more likely to be functionally important than nongenic variants. Further, variants in many genes are in low LD than those outside genes and hence may be difficult to capture by indirect association. Therefore instead of focusing on the whole genome, concentrating on the genic regions alone can provide increased coverage of the genes and also reduce the number of genotyping and burden on cost and time. The genic approach has greater power to detect variants within the genes when it suffers power for non-genic variants. Using HapMap data Jorgenson and Witte (2006) could demonstrate that it is more efficient in detecting causal variants than when whole genome approach is adopted. They suggest that combination of indirect genotyping data with gene based SNPs in high priority region

would be the best overall GWAS strategy. Alternate approach would be to use stringent LD threshold in genic regions to overcapture these regions.

The major limitation of GWAS study is the high expenditure involved to screen very large sample sizes, for getting small effects. A new approach to reduce the cost of GWAS is to conduct the study in stages/phases. In phase I a proportion of samples are genotyped for all the markers and then in phase II a proportion of these markers are genotyped in the remaining samples (Skol et al., 2007; Hastie et al., 2010).

The major issue in the study design of GWAS strategy is about the significance threshold. The conventional statistical significance determined using P-value threshold of 0.05 is not appropriate for GWAS because large number of tests performed increases the chance of type I error. Risch and Merikanga (1996) proposed new threshold levels now widely adopted which states that $P < 5 \times 10^{-8}$ which corresponds to equivalent false positive rate of 5% for 1000 000 independent tests of association. The significance of the results can be tested using Bonferroni correction i.e. by dividing 0.05 threshold by the number of tests performed. Other issues are concerned to the evaluation of statistical power of the samples to obtain valid conclusion and resolution of problems related to population stratifications.

Initial studies of GWAS have reported positive results with identification of chromosomal regions harboring disease susceptible genes but they failed to replicate in other studies because of overestimate of effective size. Other reasons were technical errors, small sample sizes, poor choice of controls and population stratification leading to type I error or false positives. These defects are rectified to certain extent using relevant corrections. National Cancer Institute and National Human Genome Research Institute Working Group on Replication in Association Studies recommended the criteria for replication. More number of GWAS are performed in the past decade identifying susceptible regions on the human chromosomes for essential hypertension.

GWAS case-control study designs may not detect the rare variants causing blood pressure variations. From 1000 genome project - an open resource catalogue of human genetic variation run by international consortium intensely genotyped samples that can be obtained to study low frequency or rare variants for fine mapping of the regions of interest. The project aims to describe over 90% of genetic variation down to 1% minor allele frequency (MAF). Next generation deep sequencing technology also facilitates the detection of rare variants needed for fine mapping.

4.3.1 Genome wide scans for hypertension

Attempts to map the genes for hypertension picked in 1990s, initially based on the study of sib-pairs, affected families and by genome wide scanning for identifying the susceptible regions. One of the earliest studies by Lifton et al. (1991) reported that mean allele sharing at the locus of sodium-hydrogen antiporter (APNH) was not greater than expected from random assortment in 93 hypertensive sib pairs.

Wellcome Trust Case Control Consortium (WTCCC), Control for Heart and Aging Research in Genome Epidemiology (CHARGE), Family Blood Pressure Programme (FBPP), British Genetics of Hypertension (BRIGHT), Global Blood Pressure Genetics (Global BPgen), Amish Family Diabetes Study (AFDS) all have reported genome wide scans results on variants

associated with variations in blood pressure and development of hypertension. Many of the initial studies could not meet the significance threshold criteria and in some chromosomal regions linkage were suggested. WTCCC reported the first genome-wide association scan results that captured 65 variants on 2000 hypertensives from unrelated participants of BRIGHT study (Caulfield et al., 2003). However the study did not reach the required statistical significance (5×10^{-7}) for hypertension and it was thought to be due to use of improper controls and inclusion of less number of tagged variants with large size effect (Barret et al., 2006; Padmanabhan et al., 2008). FBPP comprising four multi-centre network (GENOA, GenNet, HyperGen, SAPHIRE) conducted studies on different ethnic groups with different selection criteria. None of the four centers reported significant results of the genome scan and the maximum lod score obtained was 2.96 showing linkage to diastolic blood pressure on the region of chromosome 1 in Caucasians (Thiel et al., 2003). BRIGHT study (Caulfield et al., 2003) with largest homogeneous Caucasian resource with 2010 sibling pairs in 1599 families found by locus counting method a principle locus for hypertension on 6q with lod score of 3.21 that attained genome wide significance and also evidence of linkage for three other loci located on 2q, 5q and 9q. These regions did not overlap with that reported in FBPP study. Xu et al., (1999) using 367 polymorphic markers studied 200,000 Chinese adults comprising sib-pairs (207 concordant, 258 high concordant and low concordant and parents) and showed that though no regions achieved 5% genome wide significance level, regions of chromosomes 3, 11, 15, 16 and 17 were linked with lod scores of >2.0 . In their study on systolic blood pressure of 427 sibling pairs Krushkal et al., (1999) identified four regions on chromosome 2, 5, 6 and 15 with significant linkage. These regions include many potential candidate genes like phospholamban, dopamine receptor Type1A, Estrogen receptor calmodulin, sodium calcium exchanger etc. Pankaj Sharma et al., (2000) from a genome wide study identified the region on chromosome 11 to carry a new candidate gene for hypertension and suggested the need for replication of these results before attempting for positional cloning. Genome scan for blood pressure in Dutch dyslipidemic families (Hooman Allayee et al, 2001) showed suggestive evidence for linkage of diastolic BP to lipoprotein lipase gene locus on chromosome 8p. They also found evidence for linkage of systolic blood pressure to plasma apolipoprotein B levels to a locus on proximal chromosome 19p. Using 904 microsatellite markers, Kristjansson et al., (2002) reported linkage to chromosome 18q with an allele sharing lod score of 4.60 in 490 hypertensive patients belonging to 120 extended Icelandic families. Rice et al., in the same year performed genome GWS using 509 markers on 317 black individuals from 114 families and 519 white individuals from 99 families that revealed evidence for linkage ($p < 0.0023$) with base line blood pressure that replicated with other studies that located putative regions on 2p, 3p.3 and 12q.33. Von Woweren et al., (2003) from their study on genome scan of 91 Scandinavian families reported one region on chromosome 14 that reached significance threshold and 2 more regions that were suggestive of linkage with early onset hypertension. In 26 Utah pedigrees linkage of pulse pressure was suggested with loci on 8p and 12q regions by Camp et al., (2003). In a Large Chinese hypertensive kindred with 387 individuals Gong et al., (2003) found a locus on chromosome 12p that overlapped with the region containing the gene for autosomal dominant hypertension with type E brachydactyly that was mapped in large Turkish kindred (Schuster et al., 1996). This condition is now discovered as due to deletion, a reinsertion and inversion by Bahring et al., (2004). Charles et al., (2004) in their review of the GWS studies conducted earlier to 2004 summarized that all human chromosomes except for 13 and 20 have loci for blood pressure, hypertension or pre-

eclampsia. Regions on chromosomes 1,2,8,11,12,15,16,18 and 19 were replicated in more than one study and there were 3 cytogenetic intervals on chromosome 2 (2p25.3-p16.3; 2p16.1-p12; 2q23.3-q24.3) harboring loci for blood pressure that was also replicated in more than one study. Caulfield et al, (2003) associated with British Genetics of hypertension (BRIGHT) programme, phenotyped 2010 affected sibling pairs drawn from 1599 severely hypertensive families completed 10 Centi Morgan genome wide scan. Their conclusion was that human essential hypertension has an oligogenic element (with a few genes involved in the determination of the trait) possibly superimposed on more genetic effects, and that several genes may be tractable to a positional cloning strategy. In a large sample of 2959 individuals from 500 families from NHLBI, Hunt et al., (2002) identified five regions with lod score suggestive of linkage of hypertension with loci on chromosomes 1,7,12 and 15 and evidence of best linkage with a locus on chromosome 6 with systolic blood pressure. In a study of 177 Australian Caucasian hypertensive sib-pairs, Rutherford et al, (2004) showed significant excess allele sharing of D18S61 marker. At this region adenylate cyclase-activating polypeptide gene-1 (ADCYAP1) involved in vasodilation shows association with hypertension. As a complement to linkage and association studies Zhu et al, (2005) carried out admixture mapping using genome scan micro satellite markers among African Americans participating in the Lung, and Blood Institute's Family Blood Pressure Programme. Using 269 microsatellite markers the authors concluded that chromosome 6q24 and chromosome 21q21 may contain genes influencing risk for hypertension in African Americans. Kamide et al, (2005) genotyped (796 hypertensives and 1084 normotensives) 47 polymorphisms in 14 genes lying between nucleotide 8,845,292 and 11,946,689 which includes D2S2278 and D2S168 loci on chromosome 2 and concluded that GREB1 and HPCAL1 are the candidates for hypertension susceptibility. Chen et al, (2005) conducted autosomal genome scan in 775 white siblings and found suggestive linkage for total area of systolic and diastolic blood pressure on chromosome 4 and diastolic blood pressure incremental area on chromosome 18. In these regions candidate genes for hypertension like alpha and beta adducin, sodium bicarbonate co-transporter and G-protein coupled receptor kinase 4 are located.

In the recent past novel regions in the genome are localized for blood pressure and hypertension employing large number of samples and SNP markers. Org et al. (2009) studied 395,102 SNPs in 1,644 individuals from the KORA S3 cohort study, but could not find the results reaching significance level of $p \leq 10^{-8}$ for blood pressure or hypertension. The authors replicated 80 strongest associations with blood pressure and hypertension in two other European cohorts and identified a variant *CDH13* (cadherin 13 preprotein) locus (rs11646213) to be associated with hypertension and blood pressure. Newton-Chech et al., (2009) in the GBP consortium performed largest GWAS study considering blood pressure as continuous trait. They identified association of SBP with three loci (*MTHFR*, *CYP17A1* and *PLCD*), DBP with 5 loci (*FGF5*, *C10orf107*, *SH2B3*, *CYP1A2* and *ZNF 652*) at genome wide significance levels ($p < 5 \times 10^{-8}$). They also found association of 8 variants with hypertension in the same direction as blood pressure. Levy et al., (2009) performed for the CHARGE group consortium a metaanalysis of in 29,136 participants from six different cohort studies and with the population of 34,443 from GBP-gen. Their attempt revealed many novel variants for DBP and SBP with genome wide level of significance. Use of meta-analysis in the GWAS studies helps in achieving near complete genetic coverage. Meta-analysis studies by Global BPgen and CHARGE consortia have taken the number of loci to a total of 8 for

systolic, 11 for diastolic pressure and 6 for hypertension (Sajjad Rafiq et al., 2010). Yet more attempts world wide are needed to fix the genes responsible for the pathogenesis of elevated blood pressure in terms of the role played by the genes, epidemiology factors and interaction between them.

4.4 Comparative genomics

The approach of analyzing genetic basis of any disease is through comparative genomics where data from animal studies are used to extrapolate the interpretations about the mechanisms to human conditions. For human hypertension animal models offer advantages because mating and environment can be controlled. Crossing of inbred strains resolves the problem of heterogeneity. Differences in many genes like renin, Na⁺/K⁺ ATPase α subunit have been reported between hypertensive and normotensive strains of rats. Genetic polymorphisms in renin gene of Dahl rats are found to co-segregate with blood pressure in a dose dependent way supporting the involvement of the genes in the regulation of blood pressure. Similar results on co-segregation was not found for the alleles of Na⁺/K⁺ ATPase α subunit. Data from the studies on hypertensive rodent strains indicated that genes on human chromosome 17 are responsible for human hypertension. Two closely linked microsatellite markers D17S183 and D17S934 which are close to ACE gene locus were significantly associated with essential hypertension (Julier et al., 1997). The region on human chromosome 17 is syntenic to rat chromosome 10. There were also studies refuting significant linkage of hypertension to chromosome 17. Study on the translation of QTLs between rats and humans predicted 26 chromosomal regions in the human genome that are likely to contain genes controlling hypertension (Stoll et al., 2000). Many other studies on animal models helped in furthering the knowledge of conserved genomic region involved in the regulation of blood pressure and progress in this direction will improve the status of knowledge of mechanisms underlying human hypertension.

5. Future scope for hypertension management

Despite the fact that effective drugs are available, only about one out of three people has their blood pressure successfully controlled, and the blame is attributed to the undesirable side effects and the poor oral drug compliance. Keeping in mind the increasing incidence of hypertension and the patients inconsistency for the polypharmacy, immunization against renin and the angiotensins, although with less success, are being attempted. More recently, immunization against angiotensin-I with PMD-3117 vaccine, angiotensin-II with CYT006-AngQb vaccine and targeting angiotensin-II type 1A receptor with ATR12181 vaccine have provided optimism in the development of a hypertension vaccine (Pandey et al., 2009). Ang Qb vaccine has proved to become the first vaccine ever to lower (-9/-4 mm Hg) blood pressure in human beings (Pandey et al., 2009). Vaccine could induce long lasting effects with a dosing interval of months, increasing patient acceptability and compliance and thus a better control of high blood pressure. This approach has a major advantage as it can reduce the usage of medication by the patients. The results of this new biotherapy for hypertension are intriguing and promising, and vaccination for hypertension may turn out to be very useful in many patients. The impact of this treatment can revolutionise the management of essential hypertension- a disease that poses public health problems.

6. Glossary

Bonferroni correction: is a multiple-comparison correction used when several dependent or independent statistical tests are performed simultaneously

Candidate gene study: A study of specifically selected (candidate) genes in which variation is hypothesized to influence the risk of a disease.

Diastolic blood Pressure: represents the minimum pressure in the arteries when the heart is at rest.

Dominant Model: This model specifically tests the association of having at least one minor allele say 'a' (either Aa or aa) versus not having it at all (AA).

Epistatic Interaction: An epistatic interaction between two genes (non allelic) occurs when the phenotypic impact of one gene depends on another gene, often exposing a functional association between them.

Hardy-Weinburg equilibrium: An idealised state in which gene and genotypic frequencies in population do not change from generation to generation in the absence of migration, genetic drift, selection

Haplotype: is a set of closely linked genetic markers present on one chromosome of the homologous pair which tend to be inherited together and not easily separable by recombination.

Heritability: Heritability is the proportion of variation in a phenotype (trait, characteristic or physical feature) that is thought to be caused by genetic variation among individuals. It is a measure of the degree to which the variance in the distribution of a phenotype is due to genetic causes. In the broad sense it is measured by the total genetic variance divided by the total phenotypic variance. In the narrow sense it is measured by the genetic variance due to additive genes divided by the total phenotypic variance.

Hypertension (HTN): or high blood pressure is a cardiac chronic medical condition in which the systemic arterial blood pressure is elevated above the normal levels

Linkage: Linkage is the tendency of phenotypes marker alleles etc., to co-segregate in a pedigree because their determinants lie close together on a given chromosome.

Linkage Disequilibrium: Non random association of the genes present on the same or any other chromosome.

Odds Ratio: is one of a range of statistics used to assess the risk of a particular outcome (or disease) if a certain factor (or exposure) is present.

Pleiotropy: Pleiotropy is the phenomenon whereby a single gene has multiple consequences in numerous tissues. Pleiotropic effects stem from both normal and mutated genes, but those caused by mutations are often more noticeable and easier to study.

Polymorphism: Genetic Polymorphism is the presence of more than two allelic forms at a given locus in such frequencies that the rarest of them is not just due to recurring mutations but is due to a phenomenon called "polymorphisms". The frequency of the rarest allele/form as a rule is taken as $> 1.0\%$

Recessive Model: This model specifically tests the association of having the minor allele “a” as both alleles (aa) versus having at least one major allele d (Aa or AA).

Systolic Blood Pressure: represents the maximum pressure exerted in the arteries when the heart contracts.

Tagged SNPs: are representative single nucleotide polymorphisms (SNP) in a region of the genome with high linkage disequilibrium

Transmission disequilibrium test (TDT): A family-based study to compare the proportion of alleles transmitted (or inherited) from a heterozygous parent to an affected child. Any significant deviation from 0.50 in transmission ratio implies an association.

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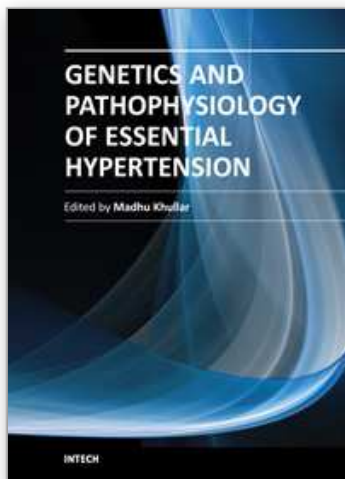
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This book, authored by renowned researchers in the field of Hypertension Research, details the state of the art knowledge in genetics, genomics and pathophysiology of Essential hypertension, specifically the genetic determinants of hypertension and role of gene variants in response to anti-hypertensive therapy. Two chapters describe mitochondrial mutations in Essential hypertension and in hypertension associated Left ventricular hypertrophy, one chapter reviews in detail the global gene expression in hypertension, and an up to date treatise on pathophysiology of resistant hypertension is detailed in another chapter. Other topics included in the book are end organ damage, baroreceptor sensitivity and role of music therapy in essential hypertension.

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