Hypertension: Molecular Approach

RD Lele

Abstract

The so-called essential hypertension is not a single entity but a mixed bag with several polygenic quantitative traits acting in concert in different combinations in different individuals. This review collates all published information from different centres using different approaches to identify candidate genes in human hypertension. 1) gene targeting approach in animal models of HT (Smithies and Maeda, 1995); 2) identification of 874 candidate SNPs in 75 candidate genes for human HT (Halushka et al, 1999); 3) comparative genomic approach translating QTLs between rat and human HT, to identify 26 chromosome regions on 16 autosomes (Stoll M et al, 2000); 4) Ten centimorgan genome-wide scan done on 2010 affected sibling pairs drawn from 1599 severely hypertensive families (Caulfield et al, 2003).

The molecular mechanisms of various molecules involved in the homeostasis of blood pressure are discussed. NO, O₂, PGI₂, EDHF, endothelin, IL-6, selectin, phospholipase A2G1B, BH4, SOD, IRS-1, adrenomedullin, PAMP, CGRP, ANP, bradykinin and bombesin; adducin α, β, γ, SAH, renin, angiotensinogen, angiotensin II, aldosterone CYP11B1, mineralocorticoid receptors, 11βHSD, DBH, PNMT, β2adrenoreceptors, and genes related to ion transport-sodium-lithium cotransporters, ENaC, NaCl cotransporters NKCC2, KCNJ and NaKATPase.

Altered gene expression in fetus due to maternal malnutrition also "programmes" for adult hypertension.

INTRODUCTION

The greatest impact of the Human Genome Project on clinical medicine is the appreciation of the extra-ordinary molecular and biochemical individuality of each patient. Gene polymorphism occurs in 1 in 1000 DNA base pairs in the human genome. This is reflected in the diversity of the gene products - structural proteins, channel proteins, transporters and binding proteins, enzymes, receptors and post-receptor signaling cascades. Polymorphism can occur not only in the protein-coding sequences but also in the upstream promoter sequences - such polymorphism can influence the activities of several enzyme-mediated processes. The most common polymorphism is single nucleotide polymorphism (SNP) - DNA sequence variation that occurs when a single nucleotide (A, T, C, or G) in the genome sequence is changed. There are on average 4 - 8 SNPs in every gene, either in the exons or coding regions (cSNPs) or in the nearby exon-intron boundaries in the upstream regulatory regions. Each person would be heterozygous for 24,000 - 40,000 nonsense (amino acid altering) substitutions.

Gene of interest can be pin-pointed using SNPs (Chakravarti, 1999). At the molecular level mutations in gene, leading to alterations in gene products or altered regulation of gene expression provide an understanding of disease. It follows logically that understanding disease at the molecular level will lead to therapy at the molecular level. New technologies such as complimentary DNA (cDNA) microarrays now available will facilitate analysis of individual variations in the whole genome and the expression profile of all genes in all types of cells and tissues, for understanding our basic genetic make up and how variations in our genetic instructions, in response to environmental influences cause disease. It is worth remembering that just as there are genes that make us susceptible to disease, there are genes that protect us from disease - this applies to all polygenic disorders such as hypertension, atherosclerosis, type 2 diabetes mellitus, neurodegenerative disorders and cancer.

With advances in genomics the search for phenotypic expression of genotype markers of diseases has increased in intensity. Every individual is a product of the interaction of his genes and the environment throughout a lifetime. Altered gene expression in the fetus can occur in the environment of maternal malnutrition, thereby contributing to the causation of hypertension.
ALTERED GENE EXPRESSION IN FETUS

Insulin receptor signaling pathways (IRS - P13K - PKB) control cellular growth in response to nutritional conditions, by the coordinated regulation of cell proliferation, cell growth and metabolism. This function is conserved during evolution and is expressed in all species including humans. Under conditions of maternal malnutrition, IRS pathways are adaptively suppressed: Low birth weight (< 2.5 kg), small head circumference and small nephron number are all consequences of this IRS pathways suppression. Brenner and Chertow (1993) showed a direct relationship between birth weight and nephron number; an inverse relationship between low birth weight and childhood, adolescent and adult blood pressure; and an inverse relationship between nephron number and blood pressure, irrespective of whether the nephron number is reduced congenitally or by acquired renal disease in later life. Decrease in nephron number and decrease in filtration surface area (FSA) may induce sodium retention. 

β-hydroxy steroid dehydrogenase (11β HSD) catalyzes the conversion of cortisol and corticosterone to inert 11 ketoproducts, normally protects the fetus from the high circulating maternal levels of glucocorticoids. Maternal protein restriction during pregnancy attenuates placental 11β HSD, and is linked to low birth weight and adult hypertension (Yang K 1997).

ESSENTIAL HYPERTENSION - NEW CLASSIFICATION

According to Ward (1990) genetic factors are responsible for about 30-60% of the familial aggregation of blood pressures and the transmission of cultural factors being responsible for the remaining (stress, diet, physical activity). Normal blood pressure is maintained by several interacting systems that control cardiac output, peripheral vascular resistance, blood volume, renal function and sodium balance.

The so called essential hypertension is not a single entity but a heterogeneous mixed bag with several polygenic quantitative trait loci (QTLs). A QTL is a chromosomal region which contains a gene or genes responsible for the quantitative trait under investigation. At the molecular level several mechanisms controlled by different genes have been identified which operate either alone (as in rare single gene mutants causing hypertension), or more commonly in different combinations in different patients. 

Arvinda Chakravarti’s group in the Centre for Human Genetics have identified 874 candidate human SNPs, in 75 candidate human genes for blood pressure homeostasis and hypertension. (Halushka et al 1999). 54% of all eSNPs lead to replacement of an amino acid residue and probably impact protein function. These SNPs are of immediate value as functional variants for blood pressure regulation and hypertension.

Candidate gene selection is based on their known or suggested involvement in blood pressure homeostasis and/or hypertension in one of the following biochemical pathways: renin-angiotensin-aldosterone; (RAA) and arginine vasopressin (AVP), - concerned with salt and water retention; neural and hormonal pathways of regulation of vascular tone, growth and repair; ion transport especially sodium and potassium; other small molecule transport pathways; regulation of glucose metabolism. A wide range of signaling molecules are involved: nitric oxide, PGI2, EDHF, ANP, endopeptidases, endothelin, bradykinin, adrenomedullin, renin, angiotensin, aldosterone, adrenaline and noradrenaline, cAMP and cGMP, phospholipases, cytokines, Ca2+, K+, etc.

Krushkal et al (1999) have found four chromosomal regions 2p, 5q, 6q, 15q linked to systolic BP in family studies of EHT.

New target regions for human hypertension via comparative genomics, based on translating QTLs between rat and human, predicted 26 chromosomal regions in the human genome that are very likely to harbour hypertension genes (Stoll M et al 2000). Five regions represent multiple blood pressure related traits- 1q, 2p13, 5q31, 15q22 and 17q. These regions on 16 autosomes represent primary targets for the development of SNPs for linkage disequilibrium studies in humans and/or provide a means to select specific models for additional functional studies and development of new therapeutic agents.

In the Medical Research Council British Genetics of Hypertension (BRIGHT) study, a 10 centimorgan genome wide scan was done on 2010 affected sibling pairs drawn from 1599 severely hypertensive families. Linkage analysis identified a principle locus on chromosome 6q with three further loci 2q, 5q and 9q showing genome wide significance when assessed under a locus counting analysis (Caulfield et al 2003). These findings imply that human EHT has an oligogenic element (a few genes may be involved in determination of the trait) possibly superimposed on more minor genetic effects, and that several genes may be tractable to a positional cloning strategy.

CENTRAL ROLE OF RENAL HANDLING OF SALT

Just as there are alleles that raise blood pressure, there are alleles that lower blood pressure. Identification of a number of genes in which mutations cause hypertension or hypotension in humans permits an assessment of the pathways by which these genes act. It is readily apparent that the final common pathway is regulation of salt reabsorption in the kidneys. Hypertension cannot be sustained without the active participation of the kidneys. Elevated renal perfusion pressure leads to salt and water diuresis returning the blood pressure to normal levels. There are inherent variations in renal salt handling. (Weinberger 1996) Transplantation of normal kidneys into genetically hypertensive recipient can prevent or correct the hypertension.

Increased renal retention of salt and water is the initiating event: intraglomerular hypertension often reflected in
microalbuminuria, which is correlated with both insulin resistance and evidence of endothelial dysfunction.

Williams and Hollenberg (1991) have divided EHT patients into modulators who handle salt load normally, with a direct relationship between salt-loading and salt excretion, and non-modulators who have impaired handling of salt-load. This non-modulating group are a phenotype seen in 40% of EHT due to increased intra-renal angiotensin II production (Williams et al. 1992).

Reduction of renal cortical nephron flow is an important and measurable phenotypic expression of the genetic basis of EHT (Britton 1981). In normotensive subjects the cortical nephron flow as a percentage of total is 84% versus 75% in EHT (due to over-constriction of afferent arterioles of cortical nephrons) ACE inhibitors like captopril are able to increase cortical nephron flow in these patients. Using ramipril, cortical nephron flow is increased from 207 ± 7 ml/min to 257 ± 21 ml/min. (Al Nahaas et al. 1990).

Alleles promoting avid salt and water retention in the salt-poor deserts (such as sub-Saharan Africa) which had survival advantage can now contribute to hypertension and its sequelae in salt-rich environment.

**Clues From Animal Models**

Although hypertension is polygenic with interaction between multiple genes among themselves and with the environment, insights into the contribution of single genes can be obtained by gene knock-out mice and transgenic mice models in which the effect of the total absence of the particular gene and the effects of increasing doses of the gene is assessed by inserting 1, 2, 3 or 4 extra copies of the gene-gene titration. Gene targeting approaches to complex genetic diseases such as atherosclerosis and essential hypertension have been used in animal models (Smithies and Maeda, 1995).

Several examples of this approach in relation to blood pressure related genes will be given in this article.

Genes implicated in hypertension in experimental animals are plausible candidates in human hypertension. A variety of genetic, environmental and demographic factors contribute to blood pressure variations even in single individuals. Only those candidate genes whose mechanism of action is understood are reviewed below.

**Review of Candidate Genes**

A. Genes related to endothelial function. (Fig. 1)

B. Genes related to RAAS - AVP.

C. Genes related to ion transport.

D. Genes related to sympathetic activity.

A. Genes related to endothelial function

1. **Endothelial nitric oxide synthase (eNOS) gene : NOS3** - Gene map Locus chrno. 7q36

   In the vascular endothelium, nitric oxide (NO) is produced by NOS using L-arginine as substrate and requiring calmodulin, NADPH and tetrahydrobiopterin (BH4) as cofactors. The single gene salt-sensitive hypertension in Dahl/Rapp rats is due to a mutant eNOS gene with deficient NO production. The hypertension can be prevented by providing excess L-arginine. Salt resistant rats respond to a dietary salt overload by producing more NO. NO is normally protective against adhesion of platelets and leucocytes, anti-inflammatory, anti-proliferative and regulates the expression and synthesis of extracellular matrix proteins.

   Endothelial dysfunction and a relative deficiency of NO is associated with some models of experimental hypertension and in human hypertension (Panza et al. 1990). Basal NO is deficient in SHRSP rats. In SHRSP model, decreased NO availability is due to excessive superoxide (O2-) generation through the NADH / NADPH oxidases, which scavenges NO to form peroxinitrite which has strong oxidant properties causing cell death. This is seen despite increase in NO synthesis (Kerr S et al. 1999). Attenuation of O2- generation can occur in the presence of DPI. Superoxide dismutase (SOD) bound to the outer surface of the endothelial cells protect NO from inactivation by O2-. The SOD mimetic tempol restores vasodilation in afferent arterioles in experimental diabetic nephropathy (Schnackenberg and Wilcox, 2001).

   Angiotensin II increases vascular O2- production through
the activation of membrane NADH / NADPH oxidases (Rajgopalan et al 1996). O2 production is greater in males than in females in animal models of HT. Estrogens cause increased scavenging of O2, as well as increased production of eNOS. Patients with uncontrolled essential HT have increased angiotensin II - mediated O2 production and increased lipid peroxidation as shown by high F2 isoprostane levels. This can be suppressed by ACEIs as well as AT1 blockers, and statins.

Endothelial NOS can constitutively produce both NO and O2. Tetrahydrobiopterin (BH4) is an important allosteric effector of NOS and plays a key role in the control of endothelial NO and O2 production in vivo. Blood vessels depleted of BH4, produce O2 because of uncoupled oxygen activation. Low BH4 levels have been associated with endothelial dysfunction in hypertension, diabetes mellitus and atherosclerosis. Abnormal biopterin metabolism is a major cause of impaired endothelium-dependent vasorelaxation and atherosclerosis. Abnormal biopterin metabolism is a major cause of impaired endothelium-dependent vasorelaxation through NO/O2 imbalance in insulin resistant rat aorta (Shinozaki et al 1999).19 Insulin stimulates the synthesis of BH4 through activation of GTP cyclohydrolase. Decreased NO-dependent vasodilation in insulin resistance is due to relative deficiency of BH4. Oral treatment for eight weeks with sepiapterin, precursor of BH4 improves endothelial dysfunction in db/db mice (single gene model of insulin resistance). It is interesting to note that ob/ob mice do not have endothelial dysfunction.

Endothelial dysfunction and insulin resistance are probably the basis of hypertension in the Indian population and hence study eNOS gene polymorphism should be a focus of special interest for us. Excessive inactivation of NO due to increased oxidative stress occurs in many clinically recognized conditions - smoking, AGEs in diabetes mellitus, oxidized LDL. Increasing NO bioactivity and prevention of NO decomposition is a primary therapeutic target by reversing endothelial dysfunction (Ruschitzka et al 1999).20 Beta blockers of the third generation such as nebivolol increase NO bio-activity, inhibit endothelin 1 liberation and vascular blockers of the third generation such as nebivolol increase NO bio-activity, inhibit endothelin 1 liberation and vascular blockers, and statins. Reduce the risk of cardiac events (Tzemos et al 2001).21 Protection of cell membrane from oxidative injury is an important function of PLA2. An association between an intronic dimorphic site in PL2G1B and hypertension has been found. EDHF (endothelium-derived hyperpolarization factor), independent of NO and prostacyclin, activates K+ channels to produce VSMC hyperpolarization and relaxation. This may be mediated through cytosolic form of PLA2 (Ca2+ dependent) releasing arachidonic acid and its metabolites EETs (epoxyeicosatrienoic acid), through gap junctions (Hutchison IR et al 1999).22 Gap junctions are channels connecting adjacent cells and mediate communication between them. Several N-acyl ethanolamines (NAEs) present in the cell membrane inhibit gap junction conductance and prevent intracellular Ca2+ signaling.

7. Insulin receptor substrate (IRS) gene : chr. 2q36 Various IRS-1 gene polymorphisms occur in normal population as well as in type 2 diabetes. A mis-sense mutation G-971-R (glycine replaced by arginine) impairs PI3 kinase activation (due to reduced association with the P85 subunit) causing insulin resistance. Mutation causing insulin resistance leads to decreased insulin-mediated NO release. Knock out mice for IRS-1 develop hypertension, hypertriglyceridemia and impaired endothelium-dependent vascular relaxation (Abe H et al 1998).23

8. Adrenomedullin (ADM) gene : chr 11p15.4 (12 SNPs) Adrenomedullin (ADM) a biologically active hypotensive peptide was discovered from human pheochromocytoma tissue in 1993 by monitoring the elevating activity of intracellular cAMP in rat platelets.

Poadrenomedullin N-terminal 20 peptide (PAMP), another biologically active peptide, was found to be processed from...
the ADM precursor (Kitamura K et al 2002).26 PAMP inhibits catecholamine secretion from sympathetic nerve endings.

ADM is found ubiquitously in tissues and organs especially kidneys, heart, lungs and blood vessels and endocrine glands. It has multifunctional biological properties to control circulation and body fluid volume regulation. Plasma ADM rapidly increases with orthostatic change in a stimulus-dependent manner and also swiftly returns to baseline after resuming supine position.

ADM is a potent vasodilator and natriuretic peptide acting through nitric oxide release. Transgenic mice with overexpression of ADM gene show greater renal NOS activity and reduced renal perfusion pressure (RPP).

DOCA- salt volume loaded hypertensive rats were treated with gene therapy with human ADM gene resulting in prolonged reduction in BP, with three-fold increase in renal blood flow and two-fold increase in GFR (Dobrzynski et al 2000).27

Adrenomedullin is prototypic of a new class of biologically active peptides, mainly expressed and secreted by non-endocrine type of cells by the stimulation with inflammation-related substances. Inflammation and hypoxia potently stimulate ADM expression and release, suggesting its unique physiological function distinct from other known biologically active peptide.

Calcitonin, calcitonin gene related peptide (CGRP), amylin and adrenomedullin are structurally related polypeptides characterized by a six or seven aminoacid ring structure linked by a disulfide bridge and an amidated C terminus. They exhibit overlapping biological actions as a result of cross-reactivity between the different receptors and G protein coupled post-receptor events. CGRP is concentrated in the locus caeruleus and has potent haemodynamic activity. Locus caeruleus is the main source of noradrenergic neurotransmission in the CNS.

B. Genes related to RAAS - AVP:

9. Renin gene : (REN) chr. 1q32 (5SNPs)

Expression of renin gene is linked up with aldosterone in a negative feed-back loop. Low renin, normal renin and high renin form a continuum in patients with EHT. When its substrate angiotensinogen is low, the kidney compensates by increasing renin production 2½ times normal thereby ensuring that all available plasma angiotensinogen will be converted to angiotensin I.

Renin is derived from its precursor prorenin. Markedly increased levels of circulating prorenin have been associated with both physiological and pathological changes. A number of extrarenal tissues such as uterine lining, ovarian theca, corpus luteum, pituitary and adrenal, express the renin gene. These tissues have different capabilities to sort and process prorenin compared with kidney, and some tissues secrete only prorenin. Whether prorenin to renin conversion is necessary to activate these local renin-angiotensin systems is a key issue. The transgenic hypertensive rat model TGR (mREN2)27 is characterized by fulminant hypertension, low plasma active renin, suppressed kidney renin and high extrarenal transgene expression in the adrenal renin and enhanced excretion of corticosteroids.

Familial hyperproreninemia is associated with normal blood pressure and normal plasma renin activity. Jeunemaitre et al (1992)28 could demonstrate no role for the renin gene in the pathogenesis of essential HT.

10. Angiotensinogen gene (AGT) (1q 42-q43) : (17 SNPs)

Angiotensinogen is a protein secreted by the liver and found in the α globulin fraction of plasma. Its sequential cleavage by renin and ACE (angiotensin-converting enzyme) produces the active hormone angiotensin II (AII) which promotes vasoconstriction. Genetically engineered angiotensinogen-deficient mice have low BP and complete absence of plasma angiotensin levels. By engineering mice carrying from zero to four copies of the angiotensinogen gene it has been shown that increased angiotensinogen levels can produce increased blood pressure. Mean angiotensinogen levels are 19% higher in blacks than in whites. In humans, a specific variant (M235T) of the gene coding for AGT has been found to segregate with hypertension in a sibling pair study (Jennemaitre X et al 1992).29 M235T (methionine replaced by threonine) mutation has been shown to be associated with EHT in white population, and with increased cardiovascular and renal risk A20C (adenine replaced by cytosine) promoter polymorphism in the AGT gene is associated with raised plasma angiotensinogen levels. High plasma levels of angiotensinogen correlate with EHT. Marker dinucleotide repeat sequence flanking the AGT gene in chr. 1q42 is strongly linked with EHT (Caulfield et al 1994).30

Brand et al (1998)31 have made an evaluation of the angiotensinogen locus in human essential hypertension. For example a heterozygous premature stop codon (Q53stop) in the gene encoding angiotensinogen leads to severe hypertension.

The estrogen component of oral contraceptive pills stimulates the hepatic synthesis of angiotensinogen.

11. Angiotensin-converting enzyme (ACE) gene : chrom. 17q23

Angiotensin-converting enzyme, or kininase II is a dipeptidyl carboxypeptidase that plays an important role in blood pressure regulation and electrolyte balance by hydrolyzing angiotensin I into angiotensin II, a potent vasoressor and aldosterone-stimulating peptide. The enzyme is also able to inactivate bradykinin, a potent vasodilator.

The ACE gene encodes two isoenzymes. The somatic ACE isoenzyme is expressed in many tissues including vascular endothelial cells, renal epithelial cells and testicular Leidig cells, whereas the testicular or germinal ACE isoenzyme is expressed only in sperms. Tissue-bound ACE is essential for the control of BP and the structure and function of the kidneys. ACE gene knockout mice show low BP, kidney dysfunction and male infertility. Mice who had a third copy
of the ACE gene and, as a result higher enzyme levels (comparable to the D/D allele) developed high BP and proteinuria. The ACE gene polymorphism and pre-disposition to high blood pressure has been shown in humans (Harrap et al 1993),32 (Stassen et al 1997).33

Besides being present as a membrane-bound enzyme on the surface of the vascular endothelial cells, ACE also circulates in the plasma (the source being synthesis in the vascular endothelium). In healthy individuals the mean plasma levels vary from 30 to 43. 50 % of the inter-individual variability of plasma ACE is determined by an insertion/deletion polymorphism (I/D) in intron 10 of the ACE gene. D/D subjects have mean plasma levels of ACE twice as much as I/I subjects. The D/D genotype is associated with left-ventricular hypertrophy, and is a useful genetic marker of LVH especially in middle-aged men and a risk factor for progression to chronic renal failure in IgA nephropathy, and predict the therapeutic efficacy of ACE inhibitors on proteinuria and progressive renal failure.

Persistant proteinuria may be related to a defective angiotensin genetically determined by the D/I polymorphism in axon 16 of the ACE gene.

The D/D genotype has greater risk for premature myocardial infarction especially due to coronary spasm and increased risk of restenosis following angioplasty.

There is evidence for a skeletal muscle RAA system and ACE I allele is associated with improved muscle performance due to increased type I skeletal muscle fibres. Post-menopausal women on HRT show significant gain in muscle power in I/I phenotype (16%) compared to D/D phenotype (7%) and also in bone mineral density.

Systolic and diastolic BP and mean arterial pressure were significantly decreased by exercise therapy in subjects with homozgyous I/I and heterozgyous I/D genotypes but not in homozgyous D/D subjects.

There is a strong linkage between circulating levels of ACE and the 17q23 region. Two polymorphism ACE4 (A240T) and ACE8 (A23506) was significantly associated with blood pressure. Rieder et al (1999)34 have discussed the sequence variations in the human ACE gene.

Interestingly an Alu polymorphism in the ACE gene Alu +/+ genotype (an Alu element insertion) exert a protective effect against age-related macular degeneration.

Familial EHT to two closely linked microsatellite markers D17S183 and D17Sq34 located on 17q, 18CM proximal to the ACE locus. The role of ACE in male fertility is completely dependent on its exclusive expression in sperm.

It is interesting to note that African-Americans show poor response to ACE inhibitors.

12. Angiotensin II receptors :
Angiotensin receptor 1 (AGTR1) : 3q21. q25
Angiotensin receptor 2 (AGTR2) : xq 22. q23
AGTR1 mediates the vasconstrictive and aldosterone secreting effects while AGTR2 regulates central nervous system functions including behaviour. AGTR2 mediates apoptosis and hence plays an important role in developmental biology and pathophysiology. Mutants lead to developmental anomalies in the kidneys and urinary tract.

13. Aldosterone synthase gene polymorphism (CYP 11 B2) (ALDOS) : chr. 8q21
Davies E et al35 have indicated the role of aldosterone synthase gene polymorphism Cyp11B2 in hypertension.

14. Mineralocorticoid receptor (MCR) : chr. not known.
A mis-sense mutation S810L -(substitution of leucine for serine) in the MCR gene makes the receptor responsive to progestosterone as well as to aldosterone. Due to altered receptor specificity, progestosterone and other steroids lacking 21 hydroxy group (normally MCR antagonist) become potent agonists. Patients with this mutation will respond to rising levels in the second trimester of pregnancy with rise in BP which comes down to normal after delivery with fall in progestrone levels (Geller et al 2000).36

15. Natriuretic peptides : ANP chr. 1q21q22 (6 SNPs), BNP chr. 1q21q13 (4 SNPs), CNP chr. 5p14p13 (9 SNPs)
Atrial natriuretic peptide (ANP), brain natriuretic peptides (BNP - also produced in cardiac ventricles) and CNP (produced in vasculature) are produced from natriuretic peptide precursors NPPA, NPPB and NPPC. ANP and BNP activate guanylyl cyclase via their receptors GC-A and GC-B. Mice with disrupted ANP gene had elevated blood pressure on standard diets. ANP counteracts the vasoconstrictor and salt and water retention effects of RAA and AVP. Many of the actions of angiotensin II are antagonized by ANP. Genetic factors leading to a decrease in ANP function are candidate causes of hypertension. Deficient responsiveness to ANP and BNP can occur due to mutations in A type receptors (vasodilatation) and B type receptors (natriuresis) (Richard RM 1994).37 Genetically reduced ANP can cause salt-sensitive hypertension.

16. Bradykinin receptor B2 (BDKRB2) chr. 14q32.1.q.32.2 (5 SNPs)
Bradykinin, an aminoacit peptide is a functional antagonist of angiotensin II. The two hormone systems are interconnected by ACE which releases vasopressor angiotensin II from its precursor angiotensin I and at the same time inactivates the vasodilator bradykinin. The AII type 1 receptor and bradykinin B2 receptor also communicate directly with each other to form stable heterodimers, causing increased activation of Gq and GoI proteins, the two major signaling proteins triggered by the AII type 1 receptor.

17. Bombesin-like receptors (BRS3) Xq26.q28 (6 SNPs)
Mice lacking functional BRS3 receptors developed mild obesity associated with hypertension and impairment of glucose metabolism. They also exhibited reduced metabolic rate, increased feeding efficiency and hyperplagia Bombesin-like peptides bind to G protein coupled receptors and modulate smooth muscle contraction exocrine and endocrine processes, metabolism and behaviour.

18. SAH (Hypertension associated SA) chr. 16p13.11
Iwai and Inagami (1991)38 identified an mRNA species that
showed markedly higher expression in kidneys of spontaneous hypertensive rats than in those of normotensive rats. SAH gene is expressed in human kidney EHT patients show three times higher frequency of allele A2 compared to normotensive persons.

C. Genes related to ion transport

19. Sodium-lithium cotransporters: chr. 17q21q22, 12p13, 3q21q25

Mutations lead to abnormal sodium transport which can be demonstrated in the red blood cells, which share the same abnormality with vascular smooth muscle cell membrane. The defect leads to an abnormal accumulation of calcium in the VSMCs resulting in a heightened responsiveness to vasoconstrictor agents. This defect may be present in 35-50% patients of EHT (Strazullo et al).39

20. Epithelial sodium channel (ENaC): α chr. 12p13, β chr. 16p13p12, γ chr. 1p36.2p36.3

ENaC is activated by aldosterone, through the mineralocorticoid receptor.

Activating mutant β or γ subunits cause excessive reabsorption of salt and hypertension (Liddle’s syndrome).

Amiloride is the effective diuretic for this condition.

Loss of function mutants (α or β subunit) cause excessive loss of NaCl from the kidneys and low BP (Pseudohypoaldosteronism type 1 - PHA 1).


22. NaK+ Cl cotransporter (NKCC2): chr. 15q15q21.1

Mutation in this gene with loss of function in this bumetanide-sensitive channel causes hypokalemic alkalosis with hypercalciuria and nephrocalcinosis (one type of Bartter’s syndrome).

23. KCNJ potassium inwardly rectifying channel - chr. 22q13.1 mutation causes renal potassium wasting (classical Bartter’s syndrome).

24. Steroid 11 β hydroxylase gene: CYP11B1 chimeric gene: chr. 8q 24

This gene and aldosterone synthase gene CYP11B2 are highly similar in DNA sequence and an identical intron-exon organization. Unequal crossing over between them gives rise to a novel gene, fusing the 5' regulatory sequence for 11 β hydroxylase onto the coding sequence of aldosterone synthase. This chimeric gene brings aldosterone synthase gene expression and enzyme activity under control of ACTH. This results in ectopic secretion of aldosterone from the adrenal zona fasciculata (rather than from zona glomerulosa under control of angiotensin II). Resultant salt and water retention and increased plasma volume leads to feed-back suppression of RAA system and reduces production of AII. However it does not turn down production of aldosterone because its secretion is now controlled by ACTH. Giving physiological doses of glucocorticoids suppress ACTH secretion which in turn suppresses the expression of the mutant gene. This is an interesting example of single gene hypertension - glucocorticoid - remediable aldosteronism (GRA).

25. 11 β Hydroxysteroid dehydrogenase: chr.1

This enzyme metabolizes cortisol to cortisone, a steroid that is incapable of activating the mineralocorticoid receptors. Cells that respond to mineralocorticoids contain this enzyme. A mutation leading to deficiency of this enzyme leads to stimulation of mineralocorticoid receptors by cortisol (inspite of very low level of aldosterone) - syndrome of apparent mineralocorticoid excess (AME).

Glycyrhrenetiac acid (derived for licorice) inhibits this enzyme to produce a syndrome similar to AME in individuals consuming large quantities of licorice.

This same mechanism of insufficient enzyme to metabolize cortisol explains the hypertension in Cushing’s syndrome and in glucocorticoid resistance caused by mutation in glucocorticoid receptor.

26. Adducin genes: α ADD1 chr. 4p16.3 (14 SNPs), βADD2 chr. 2p14p13 (8 SNPs)

Adducins are a family of cytoskeletal proteins encoded by three genes (alpha, beta, gamma) α and γ adducin are ubiquitously expressed. β adducin is expressed at high levels in the brain and haemopoietic tissue.

Both α and β adducin show alternative splicing, thus there may be several different heterodimeric and homodimeric forms of adducin, each with a different functional specificity. The name adducin comes from Latin adduere (to bring together) since adducin is thought to promote the assembly of spectrin-actin complexes in the formation of the membrane cytoskeleton. It binds to calmodulin and is an in vivo substrate for PKC.

Recent observation suggest a role for adducin in cell motility and as a target for the regulation of Rho-dependent and Ca2+ dependent pathways. Prominent physiological sites of regulation of adducin include dendritic spines of hippocampal neurons, platelets and growth cones of axons. Beta adducin deficient mouse strain (-/-) showed increased systolic and diastolic BP compared with wild-type controls (+/+). This was the first report showing direct evidence that hypertension is triggered by a mutation in the adducin gene family (Marro M et al 2000).10

The substitution of tryptophan for glycine at ammioacid 460 (Gly 460Trp) polymorphism of the alpha subunit of the heterodimeric cytoskeletal protein adducin through a modification of renal Na-K ATPase increases renal sodium reabsorption and may be involved in the pathophysiology of EHT. Intracellular erythrocyte sodium content, sodium-lithium co-transporter were significantly decreased in subjects homozygous for 460 trp polymorphism.

Casari GC et al (1995)11 have reviewed the association of the alpha adducin locus with essential hypertension. The ADD1 allele tryp 460 shows greater sensitivity to changes in
the sodium balance. Heterozygous hypertensives (gly/tryp) showed greater fall in mean arterial pressure in response to hydrochlorothiazide therapy than homozygous (gly/gly). This polymorphism may identify hypertensives responsive to diuretics alone. Alpha adducin G460w, GG and ww phenotypes were studied in relation to development of end-stage renal disease (ESRD) in hypertensive patients. ww phenotype predicted early progression (5 years) compared to GW (9 years) and GG (12 years) (Nicod et al 2002).45

The C 1797 T polymorphism of beta adducin is associated with increased risk of HT in post-menopausal women and in users of oral contraceptives particularly in the presence of the mutated alpha adducin Trp allele. Inhibition of RAAs in men and absence of such a compensatory mechanism in women may explain to some extent the sexual dimorphism of BP phenotype in relation to beta adducin polymorphism (Wang JC et al 2002).46

A new anti-hypertensive compound has been developed that can correct the abnormality of the renal Na-K ATPase and BP increase associated with adducin polymorphism in rats. Effects on humans are under evaluation (Ferrari P, Biananchi G 2000).47

D. Gene related to sympathetic activity :

27. Brain γ adducin : ADD3 chr. 4p16.3

In spontaneous hypertensive (SH) rats γ adducin gene expression was 40 - 60% lower compared to normotensive rats in the hypothalamus and brain stem (two cardioregulatory relevant brain areas) leading to two-fold increased neuronal firing and release of neurotransmitters. (adducin acts by regulating the release of neuro-transmitters. Perfusion of a gamma adducin-specific antibody caused a two fold increase in the normal firing rate, an effect similar to that observed with angiotensin II (Yang H et al 2002).45

28. Dopamine beta-hydroxylase (DBH) : chr9q34 (9SNPs):

Dopamine beta-hydroxylase (DBH) the enzyme that converts dopamine to norepinephrine and epinephrine is present in the synaptic vesicles of post-ganglionic sympathetic neurons. Release of NE is accompanied by the simultaneous release of DBH, hence plasma DBH may serve as an index of sympathetic activity.

Isolated defect of DBH causes severe postural hypotension. Plasma dopamine is elevated but DBH, NE and adrenaline are undetectable. Remarkable improvement is seen on the oral administration of DL-dihydroxyphenylserine, this agent bypasses DBH and is readily converted to NE by decarboxylation of the terminal carboxyl group.

Heavy smokes are more likely to have DBH 1368 A allele and less likely to have MAOA 1460C allele. These two enzymes help to determine the smoker’s requirement for nicotine and may explain why some patients are predisposed to tobacco addiction and why some find it very difficult to stop smoking.

Allelic association of the DBH gene with typical migraine susceptibility has been shown.

A C to T polymorphism at nucleotide 1021 in the 5′ region of the DBH gene is related to DBH levels in plasma. C/C genotype has high plasma activity (48 nmol/ml/min.); C/T genotype 25 nmol TT genotype has low activity 4 nmol.

29. Phenylethanolamine N methyl transferase (PNMT) chr 17q21-q22 :

PNMT catalyzes the synthesis of epinephrine from norepinephrine. The gene is expressed in the adrenal medulla and retina in transgene mice. This genetic locus is associated with blood pressure regulation in the stroke-prone spontaneous hypertensive rat (SHR-SP).

30. Beta-2 adrenoreceptor gene : chro 5q32q34

A polymorphism in the third intracellular loop of this receptor, which is critical for the regulation of central and peripheral sympathetic neurotransmission, occurs with a 10-fold increased frequency in African Americans (~ 40% allele frequency) compared to European Americans. Hence a drug designed to target this receptor will not be effective in a large percentage of that ethnic population. The gene encoding this receptor contains two non-synonymous coding SNPs that cause non-conservative aminoacid substitutions and affect receptor function, agonist regulation or binding affinity. The gene also contains multiple SNPs in the promoter region that affect gene expression. An association of trp64 Arg polymorphism in the β2ADR with essential HT has been noted in the sardinian population (Tonolol et al).46

Future approach to essential HT :

Within the next five years cDNA microarrays will enable clinicians to take a drop of blood from the hypertensive patient to give his gene expression profile. It will reveal polymorphism in several genes relevant to hypertension. It will also reveal other polymorphisms which will predispose the patient to diabetes mellitus, atherosclerosis and myocardial infarction. The clinician will also be able to determine if the hypertension is salt-insensitive (which means no need for salt restriction). This determination will help the choice of drugs - which drug will benefit the patient and which drugs he should stay away from. This scenario will be vastly different from the current “trial and error “method of matching a patient with a single drug or combination of drugs - thiazide diuretics, beta adrenoreceptor blockers, alpha adrenoreceptor blockers such as prazocin, calcium channel blockers, ACE inhibitors, angiotension receptor blockers, direct vasodilators such as hydralazine and minoxidil, or centrally acting drugs like clonidine and reserpine. Knowledge of polymorphic variations in the relevant genes will in future help to get the right drug for the right patient. Meanwhile, the most cost-effective drug strategy for the millions of hypertensives in India and the developing world is a combination of reserpine 0.05 mg and hydrochlorothiazide 12.5 mg, as a single daily dose, (MK Mani 2002) along with lifestyle modification : regular physical exercise, relaxation techniques and 1800 calorie vegetarian diet containing 400 gm. of vegetable and fruits - this diet is low in sodium and fat; high in potassium and magnesium, fibre content, and anti-oxidants. Dietary sources of L-arginine include water melon seeds, bhendi seeds, raddish, til and yam. Ensuring maternal nutrition and preventing low birth
weight babies will be the single most important primary prevention.

REFERENCES
Announcement

APICON 2004

Workshop-3 Clinical Epidemiology and Research Methodology, 21 Jan 2004, Time : 1000 - 1300 Hrs, Hall E

The objective of this workshop is to provide members with an introduction to plan, conduct, analyse, interpret and write up a Research Protocol. The essence of Research Methodology consists of 8 steps starting with (1) asking the correct research question, 2) Calculating the adequate sample size, 3) Selecting the sample size by proper methods, 4) Selecting the correct tools and techniques of making measurements of obtaining required information, 5) Thinking and recording information on all factors which can be indirectly associated with the study, 6) Analyzing the data appropriately using appropriate statistical tests, 7) recommendation and 8) Finally to develop new research question. This workshop is designed to provide insight into some aspects of ‘Research methodology’.

Convenor : Lt Col SP Gorthi, Delhi
Faculty : Kameshwar Prasad, Baharin
          KS Reddy, Delhi
          V Lakshmi Narayana, Chandigarh

There are limited vacancies (50-75) available. Participants are chosen on first come first served basis. There will be no registration fee. All those who are interested to participate in the Workshop are requested to communicate with the Convenor.

Lt Col SP Gorthi
Classified Specialist (Medicine and Neurology)
Base Hospital, Delhi Cantt - 110 010
Ph : (O) 011-2566 855, (R) 2566 8553, 2568 6403   E-mail : pgorthi2002@yahoo.com, Spgorthi@ndf.vsnl.net.in

Book Review

Technical Monogram - Diabetic Neuropathy

Dr. Shashank R Joshi

Diabetic Neuropathy is a very important and disabling complication of diabetes requiring much attention. The monogram ‘Diabetic Neuropathy’ written by Dr. Shashank Joshi ably deals with this condition. The book is precisely written and use of tables and algorithms have made it easily readable. The author takes the reader through various aspects of diabetic neuropathy like classification, natural history, pathology and pathogenesis to reach investigations and therapy which are dealt with great details. The common issues in diabetic foot are also discussed. This book forms a welcome addition to literature available on this subject and will be very helpful to clinicians working in various branches of medicine dealing with diabetic neuropathy.

Dr. SV Khadilkar
Hon. Asst. Prof. of Neurology, Grant Medical College and Sir JJ Group of Hospitals, Mumbai
Hon. Neurologist, Bombay Hospital, Sushrusha Hospital

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