This literature review is organized in accordance with the objectives of this study. It begins with an overview of hypertension, followed by environmental risk factors. Next, epidemiology of hypertension is considered in details along with its importance as a risk factor in cardiovascular diseases both worldwide as well as in India. Then anti-hypertensive drug prescribing patterns in different regions are explored. Hypertension, apart from leading to serious consequences, is in itself a dangerous disease which needs improvement in treatment through molecular Pharmacogenomics methods. However, given the existing Pharmacogenomics tools and the state of development of the pharmacology field, a complete strategy needs integration of gene products measured in laboratory medicine. From this perspective, one may expect a natural link between pharmacoepidemiology and pharmacogenetics in understanding unexplained variance in drug exposure and therapy outcome and developing individualized pharmacotherapy models.

Blood pressure (BP) is the outcome of the interaction of intravascular volume, cardiac output and peripheral resistance. Hypertension is defined as BP exceeding 139/89 mmHg, whereas, pre-hypertension refers to systolic BP of 120 to 139 mmHg or a diastolic BP of 80 to 89 mmHg (Fountoulakis et al., 2006).

2.1 Classification of hypertension

Traditionally, hypertension has been subdivided into two forms: essential or primary, and secondary.

Generally, hypertension or high blood pressure is classified according to its cause. The term essential hypertension refers to cases in which no specific cause can be identified (idiopathic). It accounts for approximately 85% of hypertensive patients (Fardella et al., 2000).

High blood pressure caused by another disease or condition is called secondary hypertension. It usually disappears once the underlying condition is controlled or cured. Some of the underlying conditions include sleep apnea, kidney or endocrine diseases, pregnancy, cocaine use, smoking, stress, very strenuous exercise, and long
term overuse of alcohol etc. Renal vascular hypertension is a secondary hypertension caused by kidney disease. About 15% of hypertensive patients are identified as having secondary hypertension (Thomas et al., 2007).

There are also a number of other terms that physicians use to describe high blood pressure, like malignant and labile or transient hypertension.

**Malignant or accelerated hypertension** is a sudden rise in diastolic blood pressure to over 125mmHg. This high diastolic pressure can be associated with damage to the brain, heart, eyes and kidneys (Mohan, 2005).

**Labile or transient hypertension** is a temporary rise in blood pressure during stressful situations. One very common example of labile hypertension is white coat hypertension, which occurs when people get nervous at their physician's clinic (Mancia et al., 1996). Some of the other types of hypertension are isolated and resistant. In **Isolated Systolic Hypertension (ISH)** only the systolic blood pressure is elevated, mainly in older people. Systolic pressure increases with age whereas diastolic pressure can decline after the age of 55 (Bulpitt et al., 1995).

**Resistant hypertension**, as the name suggests refers to a condition that is called so as it does not respond to typical treatments and therapies (Sheldon, 2007). It is difficult to control and often requires lifestyle changes along with a combination of medications. Persons with resistant hypertension are required to work closely with their physician, who manages their condition, and diligently follow the physician's instructions on diet, exercise and medication.

### 2.2 Diagnostic criteria for hypertension

Epidemiological evidence shows that there are several factors that play an important role in the development, evolution and prognosis of arterial hypertension. Some of them are non-modifiable, such as age, sex, ethnicity and heredity (Bianchi et al., 1979) and others modifiable, such as body weight (Stamler et al., 1997), salt intake (Beard et al., 1982), alcohol intake (Gupta et al., 1978), use of hormonal contraceptives
and drugs retaining sodium, sedentary life and psychosocial factors (Fernando, 1996). To overcome this problem, some health guidelines have been formulated for prevention, detection, treatment and control of high blood pressure as in Table: 1.

Table 1: Evolution of the Joint National Committee Recommendations for prevention, detection, treatment and control of hypertension

<table>
<thead>
<tr>
<th>Year</th>
<th>Type of Care</th>
<th>Step 1</th>
<th>Step 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>JNC-1</td>
<td>Stepped care</td>
<td>Diuretic</td>
<td>Add Metyldopa, Reserpine, or Propanol</td>
</tr>
<tr>
<td>JNC-2</td>
<td>Stepped care</td>
<td>Diuretic</td>
<td>Adrenergic-inhibiting agents (Clonidine, Metyldopa, Beta blocker, Alpha blocker, Rauwolfia)</td>
</tr>
<tr>
<td>JNC-3</td>
<td>Stepped care</td>
<td>Less than full dose of diuretic or beta blocker</td>
<td>Add small dose of adrenergic-inhibiting agent or thiazide-type diuretic</td>
</tr>
<tr>
<td>JNC-4</td>
<td>Individualized Stepped care</td>
<td>Diuretic, beta blocker, calcium channel blocker, or ACE inhibitor</td>
<td>Add second drug of different class., increase dose of first drug, or substitute drug of different class</td>
</tr>
<tr>
<td>JNC-5</td>
<td>Modified stepped care</td>
<td>Diuretic or beta blocker alternative therapy: ACE inhibitor, CCB, beta blocker, alpha blocker</td>
<td>Increase dose or substitute another drug, or add a second agent from a different class</td>
</tr>
<tr>
<td>JNC-6</td>
<td></td>
<td>Uncomplicated hypertension: diuretic, beta blocker</td>
<td>Specific indications for ACE inhibitor, ARB, alpha-beta blocker, beta blocker, CCB, and diuretic substitute another drug or add second agent low-dose combination therapy may be appropriate initial therapy</td>
</tr>
<tr>
<td>JNC-7</td>
<td></td>
<td>Thiazide diuretics for most, may consider ACEI, ARB, BB, CCB</td>
<td>Two Drug combination mostly prescribed</td>
</tr>
</tbody>
</table>
2.2.1 JNC-7 Blood pressure classification (Chobanian et al., 2003)

<table>
<thead>
<tr>
<th>BP Classification</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;120</td>
<td>&lt;80</td>
</tr>
<tr>
<td>Prehypertension</td>
<td>120-139</td>
<td>80-89</td>
</tr>
<tr>
<td>Stage-1 Hypertension</td>
<td>140-159</td>
<td>90-99</td>
</tr>
<tr>
<td>Stage-2</td>
<td>≥ 160</td>
<td>≥ 100</td>
</tr>
</tbody>
</table>

SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure

Recommendation for follow-up based on initial blood pressure measurements for adults. (Chobanian et al., 2003)

<table>
<thead>
<tr>
<th>Initial BP (mm Hg)</th>
<th>Follow-up recommended to confirm diagnosis and/or review response to treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;130</td>
<td>&lt;85 Recheck in one year</td>
</tr>
<tr>
<td>130-139</td>
<td>85-89 Recheck within 3-6 months</td>
</tr>
<tr>
<td>140-159</td>
<td>90-99 Confirm within two months</td>
</tr>
<tr>
<td>160-179</td>
<td>100-109 Evaluate within one month and treat if confirmed</td>
</tr>
<tr>
<td>180-209</td>
<td>110-119 Evaluate within one week and treat if confirmed</td>
</tr>
<tr>
<td>≥ 210</td>
<td>≥ 120 Initiate drug treatment immediately</td>
</tr>
</tbody>
</table>
Evaluation of patients with documented essential hypertension has three objectives:

1. To exclude secondary causes of hypertension
2. To ascertain the presence or absence of target organ damage.
3. To assess the lifestyle and identify other cardiovascular risk factors or concomitant disorders that affect risk factors, prognosis and guide treatment.

### 2.2.2 Secondary causes of hypertension

Sleep apnoea, drug use, chronic kidney disease, primary aldosteronism, renovascular disease, chronic steroid therapy and Cushing syndrome, phaeochromocytoma, acromegaly, thyroid or parathyroid disease, coarctation of the aorta and takayasu arteritis (Simons et al., 2003).

### 2.2.3 Cardiovascular risk factors

Major risk factors are: Hypertension, cigarette smoking, central obesity (waist circumference >90 cm for men, >80 cm for women), physical inactivity, dyslipidaemia, diabetes mellitus, microalbuminuria or estimated GFR <60ml/min, Age older than 55 years for men & 65 years for women, family history of premature cardiovascular disease (men<55 years or women <65 years) (Haq et al., 1997).

### 2.2.4 Target organ damage

Heart: left ventricular hypertrophy, angina or prior myocardial infarction, prior coronary revascularization, heart failure (Obrien et al., 1995)

Brain: stroke or transient ischemic attack (Lim et al., 2000)

Chronic kidney disease, retinopathy (Lim et al., 2000)

The information is obtained from adequate history, physical examination, laboratory investigations and other procedures.
2.2.5 History of the disease

Duration and level of elevated BP (if known), symptoms of secondary causes of hypertension, symptoms of target organ damage, family history of hypertension, dietary history, drug history and lifestyle environment factors.

2.2.6 Physical examination

General examination, including height, weight and waist circumference, measurement of BP on both arms.

2.2.7 Initial investigations to exclude secondary hypertension

Full blood count, urine analysis, measurement of urine albumin excretion, renal function tests, fasting blood sugar, lipid profile.

2.3 Hypertension: a complex condition

The interplay between environmental and genetic risk factors leads to the development of intermediate phenotypes, such as obesity and insulin resistance (Maolian et al., 2006). Hypertension may be understood as a final phenotype resulting from a number of intermediate phenotypes. In terms of molecular biology hypertension can be defined as a complex, polygenic, multifactorial condition. Each patient may present different causative environmental and/or genetic factors for the trait. (Krieger et al., 2006).

Blood pressure (BP) is the result of cardiac debit and peripheral vascular resistance. New systems and mechanisms affecting cardiac debit and peripheral vascular resistance have given us an insight into a complex chain of physiopathological inter-relations leading to hypertension (Rigatto et al., 2004). Changes in the renal retention of sodium, sympathetic nervous system, rennin-angiotensin system (RAS), cell membrane, and hyperinsulinemia are integral parts of this complex physiopathological chain (Irigoyen et al., 2003). An understanding of each of these components as intermediate multiple-gene phenotypes supports the concept of hypertension as a complex condition.
Many studies have been conducted to identify hypertension susceptibility genes (Table 2). Still, the number of genes involved is not known; neither are the transmission modes, quantitative effect on BP, interaction with other genes or with environmental factors (Rubbert et al., 2003). Genetic factors are considered to be responsible for one third of all factors involved in hypertension etiopathogeny (Barreto et al., 2003; Fava et al., 2004; Poch et al., 2001 and Ruppert et al., 2003). However, many studies may have underestimated the impact of genes since behavioral patterns such as obesity and alcohol abuse are modulated by genetic factors (Krieger et al., 2006). Response to physical exercise has been demonstrated to vary from individual to individual, thus suggesting that the effects from exercise may be mediated to a great extent by genetic variations (Oliveira et al., 2003).

The study of hypertension molecular determinants is made more complex not only by the gene-environment interaction, but also by the interference of the multiples alleles themselves, which individually may have little influence on final phenotype but may have significantly additional effect when combined (Castellano, 2004; Doris, 2002 and Luft, 2004). Although a high correlation between combined RAS-related genotypes and prevalence of hypertension has been reported, no individual effect of each isolated genotype has been detected (Siani et al., 2004). On the other hand, some hypertension genes may be activated at certain points of time. Therefore, some individuals who develop hypertension at a later age may have hypertension gene activation as the mechanism responsible. Despite such evidence, longitudinal studies are needed to investigate the association between hypertension and genes at different age ranges (Mondry et al., 2005). Mutations at different loci in the same gene may also make the understanding of the impact of genes on hypertension even harder (Rubbert et al., 2003).

However, it is not only these aspects that make the study of genetic polymorphisms associated with hypertension more difficult. There are limitations of study methodology. Most of the commonly-used studies have a case-control design and linkage analysis (Bloch et al., 2006). Case control studies screen non-related individuals, allow larger samples and have higher statistical power, but are more susceptible to false positives. Linkage analyses recruit individuals within the same
family or groups of family members who have a history of hypertension. As a smaller number of individuals are enrolled in the study, power is reduced (Irigoyan et al., 2003).

Table 2: Systems/Mechanisms involved in the Pathophysiology of hypertension and hypertension susceptibility candidate genes (Sandro et al., 2007)

<table>
<thead>
<tr>
<th>System/Mechanism involved in hypertension pathophysiology</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renin-Angiotensin System</td>
<td>Renin (REN)</td>
</tr>
<tr>
<td></td>
<td>Angiotensinogen (AGT)</td>
</tr>
<tr>
<td></td>
<td>Angiotensin Converting Enzyme (ACE)</td>
</tr>
<tr>
<td></td>
<td>Angiotensin II AT1 Receptor (AGTR1)</td>
</tr>
<tr>
<td></td>
<td>Angiotensin II AT2 Receptor (AGTR2)</td>
</tr>
<tr>
<td>Sympathetic Nervous System</td>
<td>β1 Adrenergic Receptor (ADRB1C)</td>
</tr>
<tr>
<td></td>
<td>β2 Adrenergic Receptor (ADRB2C)</td>
</tr>
<tr>
<td>Ions Transport</td>
<td>Subunit B3 of protein G (GNB3)</td>
</tr>
<tr>
<td>Na transport</td>
<td>Alpha adducin (ADD1)</td>
</tr>
</tbody>
</table>

2.4 Pathophysiology of hypertension

There is still much uncertainty about the pathophysiology of hypertension. A small number of patients (between 2 and 5%) have an identifiable cause for their raised BP, such as renal or adrenal disease (Sinclair et al., 1987). However, majority of cases have no clear single identifiable cause and their condition is labeled as “essential hypertension”.

A number of physiological mechanisms are involved in the maintenance of normal BP and their derangement may play a part in the development of essential hypertension. It is probable that a great many interrelated factors contribute to raise BP in hypertensive patients, and their relative roles may differ among individuals.
2.4.1 The Rennin-Angiotensinogen System

Rennin is responsible for converting angiotensinogen to angiotensin I (Ang I), a physiologically inactive substance which is rapidly converted to angiotensin II (Ang-II) by ACE in tissues such as the lungs (Cain et al., 2002). ACE is also responsible for the catabolism of various biologically important peptides (e.g., bradykinin) into inactive metabolites. Ang II is a potent vasoconstrictor and thus raises BP. In addition, it stimulates the release of aldosterone from the zona glomerulosa of the adrenal gland, which results in further rise in BP due to Na⁺ and water retention (Unger, 2002).

The circulating Renin-angiotensin-aldosterone system (RAAS) is not thought to be directly responsible for the rise in BP in essential hypertension (Cain et al., 2002). In particular, many hypertensive patients have low levels of rennin and Ang-II (especially elderly and black people) (Schmaier, 2003). There is, however, increasing evidence that there are important non-circulating local ACE-independent rennin-angiotensin epicrine or paracrine systems, which also control BP (Lavoie et al., 2003). Angiotensinogen can be converted directly to Ang-II by enzymes such as tissue plasminogen activator, cathepsin G, and tonin, while chymostatin-sensitive Ang-II-generating enzymes chymase and cathepsin G are able to catalyze the hydrolysis of Ang I to Ang-II (Johnston et al., 1997). Local rennin systems have been reported in the kidney, the heart, and the arterial tree.

2.4.2 The Adrenergic System

Catecholamines exert cellular action via binding to adrenoreceptors through G-proteins. Studies have revealed many subtypes of adrenoreceptors such as the Û₁a, Û₁b, of the Û receptors, the Û₁, Û₂, Û₃, Û₄, Û₅, and Û₆ types of the Û receptors, and ß₁, ß₂, and ß₃ of the ß receptors (Flordellis et al., 2004).

Although there is growing evidence that essential hypertension is commonly neurogenic and is initiated and sustained by hyperactivity of the sympathetic nervous system, the precise casual mechanisms leading to sympathetic augmentation in hypertensive subjects are still not entirely clear (Schlaich et al., 2004). Other possible mechanisms include increased sympathetic nerve firing rates, altered neuronal norepinephrine reuptake, diminished arterial baroreflex buffering of sympathetic nerve traffic, and facilitation of norepinephrine release by neurohumoral factors such as Ang-
II (Schlaich et al., 2003). An increased sympathetic activity is thought to, at least in part, both initiate and sustain the elevation of BP. High renal sympathetic tone contributes to development of hypertension by stimulating rennin secretion and promoting renal tubular reabsorption of Na⁺. Catecholamines are an important factor, not least due to the fact that the drugs that block the sympathetic nervous system do lower BP and have a well established therapeutic role. Hypertension is probably related to an interaction between the autonomic nervous system and the RAAS, together with other factors like Na⁺ reabsorption and circulating volume (Esler, 2002).

2.4.3 Renal Na⁺ handling

Many mechanisms affecting Na⁺ transport are involved in the maintenance of a normal BP. Human renal transplant studies show that there is a genetic component to renal factors that mediate essential hypertension (Guidi et al., 1996). For example, previously normotensives renal transplant recipients without a family history of essential hypertension who receive a kidney from a donor with a family history of essential hypertension develop hypertension more frequently and require more medication for BP control (Guidi et al., 1996). This is not so when they receive kidney from a donor without family history of hypertension.

Data suggest that Na⁺ intake in excess of that needed to maintain normal extra cellular fluid volume is necessary but not sufficient for hypertension (Weinberger 1996). Many subjects with essential Hypertension do not reduce BP in response to dietary Na⁺ restriction (Ben-dov et al., 2011). Mechanism other than Na⁺ intake must mediate their high BP. Some investigators postulate that narrowing of afferent arterioles in some nephrons leads to higher perfusion pressure which in turn release more vasoconstrictors such as Ang II (Sealey et al., 1988). This phenomenon would cause vasoconstriction in adjacent normal glomeruli due to local release of Ang-II and possibly other vasoconstrictors, and due to increased perfusion pressure to all afferent arterioles because of the increased systemic BP. Some studies suggest that an excess of other vasoconstricting substances or a deficit of vasodilating substances contribute to essential Hypertension in some subjects (Soundararajan et al., 2010). Some subjects with essential Hypertension have increased plasma levels of arginine vasopressin (Tarif,
Selective inhibition of the V1 receptor reduced BP in these individuals, supporting a causal role of this mechanism in their HT. Still other studies show that hypertension itself reduced tonic release of NO, which is a relaxant. Some subjects with essential hypertension had a primary defect in agonist-induced NO (Cho et al., 1999).

Several physiological, biochemical, and anatomical traits contribute to an individual’s blood pressure level, which is homeostatically maintained through a complex interaction of interrelated systems that exert reductant and counterbalancing pressor and depressor effects (Guyton, 1981). In normotensives as well as hypertensive individuals, cardiac output (heart rate x stroke volume) and peripheral resistance are controlled by overlapping control mechanisms, i.e. the baroreflex mediated by the sympathetic nervous system, parasympathetic nervous system, and the rennin-angiotensin system.

2.5 Global prevalence of hypertension

Cardiovascular disease is now endemic worldwide and is no longer limited to economically developed countries (Lawes et al., 2008). India, with the second largest population in the world, faces huge challenges in providing its citizens with necessary basic facilities and health care (Radhakrishnan and Jospeh, 2008). More than 70% of the population lives in over 5,50,000 villages and the remainder in nearly 200 urban towns and cities (Ranganathan et al., 2005). The estimated prevalence of coronary artery disease in India is currently about 3% to 4% in rural areas and 8% to 10% in urban zones (Radhakrishnan and Jospeh, 2008). In recent years, rapidly increasing economic and demographic changes in many developing countries are resulting in a shift of attention from infectious diseases to non communicable diseases (Reid and Thrift, 2005).

Hypertension is an established major risk factor and the leading cause of cardiovascular disease worldwide. The incidence of hypertension is decreasing in the developed countries whereas it is increasing in the developing countries. Its prevalence depends on both the genetic composition of the population and the criteria used to define the condition. It varies from country to country, population to population and rural to urban area. In general, prevalence of hypertension increases with age and is
higher in blacks than in whites (Gupta, 1997). A study found the prevalence to be 37.7% in Italy, 38.4% in Sweden, 41.7% in England, 48.7% in Finland, 46.8% in Spain, 55.3% in Germany, 20-33% in Africa, and 25-30% in China, Korea, and Taiwan. The mean prevalence of hypertension was higher in Europe at approximately 44% than in the US at approximately 28% (Wolf-Maier et al., 2003).

Various studies have found hypertension in adults ranging from 3.15% to 40.0% (Pearson et al., 2002 and Piccini, 1994). Studies conducted in Turkey have shown that women have a significantly higher prevalence than men (34.1% and 15.6%, respectively).

Cardiovascular diseases were estimated to have led to 1.59 million deaths in India in the year 2000 and this figure is projected to increase to 2.03 million for the year 2010 (Ghaffar et al., 2004). Nearly 40 million people in India are hypertensive and more than 62.5% of these show no apparent cause and are diagnosed as essential hypertensive (Gupta, 1997).

In 2000, an estimated 972 million adults were hypertensive. Of these, 333 million were living in economically developed countries (Kearney et al., 2005) and 639 million in economically developing countries.

By 2025 the number of hypertensive people in the world is projected to increase by about 60% to a total of 1.56 billion, as the proportion of elderly people will increase significantly. Other reasons are the continuing population increase and lifestyle changes such as diet made of processed foods rich in sugars and fats and sedentary living made possible by televisions, computers and cars (Maron et al., 2006).

Polpinit et al (1992) reported that the prevalence of hypertension and diastolic hypertension increased gradually with advancing age, while the isolated systolic hypertension increased after the age of 60 years in rural area of Khon Kaen. Hypertension was more prevalent in non-farmers than in farmers. Overall prevalence to be 11.1% in south-western Saudi Arabia reported Mostafa et al. (1996). The age-adjusted prevalence was 10.6% in men and 11.4% in women, which increased significantly with age.
The statistically significant differences in prevalence of hypertension was measured in different regions of Saudi Arabia reported (Mansour et al., 1998). Highest prevalence was found in Farasan (8.9%) and lowest in the Asir region (2.2%), which is a pattern similar to the affluent western countries.

A study on the Dutch elderly people showed the prevalence of hypertension to be more in women than in men which increased with age (Van-Rossum et al., 2000). However, there were no significant differences in BMI, smoking, and alcohol intake between these populations for men under 60 years and women under 30 years of age. Duhot et al (2000) found the prevalence of hypertension at 10.73% which was more in women than in men and increased with age.

A study of Saudi Arabian mixed community showed higher prevalence of 29.86% among females in the age group of 65 to 74 years and 30.3% in the age group above 75 years, compared to prevalence in males at 19.67% and 17.02%, respectively (Siddiqui et al., 2000). This was attributed to various lifestyle factors and poor literacy rate. The study also recommended modification in the existing lifestyle. Similar association was also reported from Japan and China (Ueshima et al., 2000). The prevalence in rural areas was found higher than in urban areas, which was attributed to high fat diet, high rate of obesity, heavy alcohol drinking and sedentary lifestyle.

The overall prevalence of 43% among elderly people in Mexico (Gracia-Pena et al. 2001), was much higher compared to earlier studies which found it to be 4.7% to 29.2% (Caamano et al., 1982; Rodriguez et al., 1982). The prevalence was higher among females than males. They found no significant difference in the mean age or educational level between the groups. The higher prevalence in females was attributed to higher stress.

Singh et al. (2002) found 30.8% of Jamaican adult population to be hypertensive. A remarkable finding of this survey was the possible role of family history of hypertension among the black people who made 95% of the Jamaican population. Narvez et al (2001) found the overall prevalence of hypertension in Spain (58.6%) very similar to the one reported (60%) by Anderson et al. (1972). However, when the data was studied according to JNC-7 criteria, a higher prevalence of
hypertension (77.8%) in elderly women was observed compared to other studies where moderately lower prevalence of 61.3% and 74.2% have been recorded.

Ordunez et al (2001) showed the prevalence in the Latin American and Caribbean ranging from 8% to 30%. Another study showed higher prevalence of hypertension among Afro-Caribbean men and women compared to Caucasians but similar to Asian men (Lane et al., 2002). In China, adult hypertensive population between the ages of 34 and 74 years increased from 30 million in 1960 to 94 million in 1990 and 130 million in 2002 (Yangfeng et al., 2008). The prevalence of hypertension in China exceeded many developing industrialized countries.

Duprez et al (2002) studied working adult Belgian population and reported the blood pressure in one third of the men and one quarter of the women with strong association between age and systolic blood pressure in both the gender.

The summary report about South American population documented 6 times higher risk of hypertension among individuals with elevated cholesterol levels Pramparo et al (2002). A health survey in England reported the prevalence of hypertension to be 3.3% in those aged under 40 years, 27.9% among those aged between 40 and 79 years and 49.9% in those over 80 years. Similar figures are seen throughout the developed world (Khan et al., 2005).

Raganathan et al (2005) reported that three decades ago, prevalence of cardiovascular disease in India was higher than other Asian countries (Japan, China, and Taiwan) but compared to the United States it was four times higher possibly due to genetic determinants mediating by serum lipid levels. The overall prevalence of hypertension in the urban population of Brazil was reported at 22.58% and found the positive association between with body mass index and blood pressure (Feijao et al., 2005).

A study in rural Peshawar reported overall prevalence of hypertension at 38% (Hassan et al., 2005). It was more in females (41.1%) compared to males (32.8%). The dominant risk factors were physical inactivity (64.3%), followed by increased BMI (54.6%). The overall prevalence of hypertension in the south west Nigerian population
was 21% and it depended significantly on age, body mass index and gender (Erhun et al., 2005). The mean blood pressure of East Asian population of South East Asian countries was generally found to be lower than the Europeans but at same levels as north Americans, and was strongly associated with body mass index and blood pressure (Harrison et al., 2006).

A study in New Jersey found that the prevalence of hypertension increased by 18% from 1999 to 2005. The increase was more in the population above 60 years of age (Carzine et al., 2006). Pitsavos et al (2006) found the prevalence of hypertension in Greek population of Greece was higher in women than in men and showed a strong correlation between hypertension and diet.

Shapo et al. (2003) found that men were 63% more likely to be hypertensive than women and that its prevalence increased with age among both sexes: between ages 55 to 64 in men and above 65 in women.

Chen et al. (1995) surveyed isolated systolic blood pressure in the Kinmen islands of China and found its prevalence 41.9% in men and 29.9% in women above 30 years of age. The prevalence increased rapidly with advancing age in both sexes. Men had lower awareness of hypertension than women.

A study on the Pakistani population from 1990-1994 found the overall prevalence of hypertension at 25% (Jafar et al., 2006). The prevalence was highest in men in the age group of 35-54 years and in women in the age group of 15-24 years. This result was opposite to the findings on the Malaysian population where Lim et al (2004) reported 23% prevalence which was higher in younger men than in younger women and the reverse in older men and women among the Chinese and the Indians. The rate was higher in Malaysian women than in Chinese and Indian women.

The self reported prevalence of hypertension in the Taiwanese population was found to be 31.1% for men and 38% in women which is associated with gender, age and BMI, but not with cigarette smoking and alcohol-drinking (Tsai et al., 2007). Arslantas et al (2008) reported overall prevalence of hypertension at 59.5% in western Turkey.
Many studies conducted in Turkey have shown higher prevalence among women than among men.

In the Canadian population, Karen et al. (2008) reported that the prevalence of hypertension increased by 60% between 1995 and 2005, a statistically significant result recorded after multivariable adjustments for age and sex.

The prevalence of hypertension in the Iranian population was found to be strongly age-dependent (Haqhdoost et al., 2008). It was 23% in the middle age group (30-55) and 49.5% in older age group (>55). The prevalence in females was around 13% higher than in males, which is consistent with other studies on the Iranian population.

The prevalence of hypertension among school-age children who were overweighted was the subject of another study (Jonathan et al., 2004). They observed that hypertension varied significantly with ethnicity (31% Hispanic, 20% African American, 15% whites, and 11% Asian).

The dependence of hypertension on age and body mass index went up from 24.4% to 28.9% among non-Hispanic women. These observations were made by the United States national health and nutrition surveys of adult population from 1988 to 1994 and 1999 to 2004 (Jeffrey et al., 2008).

The study reported there was strong association between hypertension and bmi rather than age in essential hypertensive of Peshawar, Pakistan population. Increased prevalence of hypertension with advancing age was also observed (Humayun et al., 2009).

The overall prevalence of hypertension was observed 11.5% in adult population of Saudi Arabia (Hamdan et al., 2010). Among these, there were more number of females compared to males which were similar studies in Arab and Muslim Countries. They also observed the strong relation of hypertension with advancing age and other contradictory study (Dorobantu et al., 2010) reported on Romania population that more number of males (50.17%) were observed compared to females (41.11%). The overall
prevalence of hypertension (44.92%) in which greater prevalence was seen in rural area (49.47%) than urban area (41.58%).

2.6 Epidemiology of hypertension-- an Indian scenario

Cardiovascular diseases are a major public health threat and a growing clinical problem among Asian Indians (Mohan et al., 2001). These diseases are estimated to have led to 1.59 million deaths in India in the year 2000 and the figure is projected to increase to 2.03 million by the year 2010 (Ghaffar et al., 2004). This study, which is also known as the Global Burden of Diseases study, suggests that by the year 2020, India will have more individuals with cardiovascular diseases than other regions of the world. Since the 1950s, the prevalence of cardiovascular diseases has increased significantly among the Asian Indians. The increase is significantly greater in the urban than in the rural areas. Pooling of epidemiological studies shows that hypertension is present in at least 25% urban and 10% rural adults in India (Mohan, 2006). Various studies have estimated a prevalence ranging from 1.24% in 1949 to 36.4% in 2003 among urban population and from 1.99% in 1958 to 21.2% in 1994 among rural population (Gupta, 1997).

Dubey et al (1954) carried out one of the earliest studies in India and reported a prevalence at 4% (criteria: >160/95mmHg) amongst industrial workers of Kanpur. In 1984, Wasir et al 1984 reported 3% prevalence of hypertension (criteria: ≥160/95mmHg) in Delhi. During 1984-87, the prevalence of hypertension in Delhi (criteria: ≥160/90mmHg) was found to be 11% among males and 12% among females in the urban areas and 4% and 3% respectively in the rural areas (Chadha et al., 1997). Two other studies in the urban areas of Haryana (1994-95) found 4.5% prevalence (JNC-5 criteria) while urban areas of Delhi showed a prevalence of 45% in 1996-97.

An ICMR study in 1994 on 5537 individuals (3050 urban and 2487 rural) showed 25% and 29% prevalence of hypertension (criteria: Ô140/90mmHg) among males and females, respectively in urban Delhi and 13% and 10% in rural Haryana. Kalavathy et al (2000) reported the prevalence of hypertension to be higher at 51.8% in Kerala, a state with maximum number of elderly people among Indian states.
Zachariah et al. (2003) reported the prevalence at 54.5% in the middle-aged urban Keralites, which was the same for both the sexes. Awareness of hypertension did not differ among men and women due to high level of education among both sexes in Kerala.

Prevalence of hypertension in the Parsi community was found to be 35.8% in rural areas and 32% in urban areas. The same study found the prevalence of hypertension in the general caste population to be significantly higher than in the Scheduled Castes (Karkal, 1983). A similar trend was found in widows vs. married and illiterates vs. educated subjects. But there was no significant difference among sexes (Bharucha et al., 2003).

Gupta (2004) through three serial epidemiological studies (criteria: \( \geq 140/90 \)) carried out in Jaipur in 1994, 2001 and 2003, demonstrated rising prevalence of hypertension (30%, 36% and 51% respectively among males and 34%, 38% and 51% among females).

Rural population of Assam had a prevalence of 33.3% in men and 33.4% in women (Hazarika et al., 2004). Age and extra salt intake was a significant risk factor for hypertension among both sexes. Smoking and heavy drinking were more frequent in men (20%) than in women (1.9%).

The prevalence recorded School-going children (age 11-17 years) had prevalence of 6.69% in urban and 2.56% in rural areas (Mohan et al., 2004). The difference was due to more overweight and obese children in the urban areas. No gender difference was found. The rural-urban differences probably reflect the changing lifestyle and environmental interactions. None of rural students with normal BMI was found to be hypertensive, which support the relationship between hypertension and obesity. Haldiya et al. (2004) selected two places, based on the changes in dietary habits like consumption of salt, edible oil and ghee, and found prevalence of hypertension to be over 20% in Ghachipur and 10.4% in Balarwa.

The overall prevalence of hypertension was found to be 24.9% using JNC-7 criteria. Men showed high prevalence of both systolic and diastolic hypertension at
young age but women displayed higher prevalence of diastolic hypertension after 40 years of age (Das et al., 2005). This was attributed to increasing family stress and obesity, which is common among middle-aged women.

The overall prevalence of hypertension found 20.6% (Deshmukh et al., 2005) in Maharashtra. It was 21.8% among males and 19.8% among females, compared to 2.8% among males and 4% among females in rural Maharashtra in 1993. They further observed that the risk of hypertension increased significantly with an increase in age, BMI and waist hip ratio.

Yadav et al (2008) reported an overall prevalence of hypertension in Lucknow 32.2% compared to different earlier studies which reported 5.2% in Agra 1960-1980 (Mathur et al., 1963). The prevalence increased significantly from the age groups of 30-39 to 60-69. Body mass index and sedentary lifestyle were both associated with metabolic risk factors to increase the incidence of hypertension. Smoking and disturbance of dietary habits play a major role in hypertension (Singh et al., 2006).

Apart from age, employment is a significant factor among residents of Indian cities. In India, significantly lesser proportion of women are employed, and married women may not have access to their own income. Physical inactivity led to increased body weight and central deposition of fat, which reflected in the waist girth and BMI (Sharma et al., 2006). Risk factors of hypertension in coronary artery disease were high in both rural as well as urban population of India (Banarjee et al., 2006). They are widely prevalent in the Indian population, irrespective of cultural or socioeconomic differences. There was direct association of hypertension with BMI and geographical areas of different regions reported (Krishna et al., 2007).

The prevalence of hypertension has almost doubled in Chandigarh over the past 30 years (Kar et al., 2007). This study showed the prevalence of self reported hypertension to be 32.3%. Physical inactivity and accumulation of body fat made the Chandigarh population highly vulnerable to cardiovascular morbidity and mortality.

Risk of hypertension in urban south India (Chennai) was found to increase with higher salt intake than recommended by WHO (Radhika et al., 2007). Similarly, a
strong association of hypertension with salt intake was recorded in urban North Kashmir whereas no such association was found in urban residents of Kerala.

Prasanth et al (2008) studied the prevalence among Keralite population and found it to be 33.5% which was much higher compared to an earlier study which put it at 8.8%.

A study from Jaipur found body mass index to be an appropriate predictor of hypertension (Gupta et al., 2009). The result is consistent with other studies done in North America, Europe and Asia which showed significant linear correlation of dietary fat intake with body mass index and blood pressure (Enas et al., 1996).

According to JNC-6 criteria (Yadav 2008) studied, to measure the overall prevalence of hypertension in resettlement colony of Delhi which was 39.5%, with slightly higher rate (41%) in males than in females (38%).

The prevalence of systolic hypertension to be 18.5% and diastolic hypertension to be 15% in the rural population of 60 years and above found through (Agrawal 2008). There was a significant correlation between systolic hypertension and age in men but the difference was not statistically significant in women.

The prevalence of systemic hypertension among the rural Keralites investigated and found 33.5% of the subjects to be hypertensive (Prasanth et al., 2008). Out of which 64.3% among females and 35.7% among males. As the age increases from 20 to 49 years, the prevalence among men is higher but after wards women overtake men.

The prevalence of hypertension was observed 26.5% due to body mass index and waist circumference in Chennai, South Indian (Kaur et al., 2008)

Chandwani et al (2010) reported the prevalence of hypertension was 24% among the urban area of Jamnagar, Gujarat. High prevalence was found among non-vegetarian males with BMI greater than 25.

In rural area of Rajasthan, the high prevalence of hypertension was observed (23.1%) due to various risk factors like ghee consumption, lifestyle and tobacco (Haldiya et al., 2010).

Biswa et al., (2010) reported the risk factors of essential hypertension in West Bengal population. They observed high level of total cholesterol and triglyceride were consumed in essential hypertensive compared to normotensives population other study
reported in urban community of West Bengal, India (Mandal et al., 2010). They observed that prevalence of hypertension was 19.80%, among these with advancing age more number of females compared to males. 76.7% risk factor in this population due to body mass index, waist circumference, smoking, high consumption of cholesterol.

2.7 Prescription patterns of antihypertensive drug therapy

Drug therapy is the most commonly used method for treatment of any disease. General practice databases have been used as an effective method for pharmacoepidemiological research. Research on pharmacoepidemiology to develop quality control programs in general practice and formulate guidelines for the proper use of drugs is an obvious need and challenge for practitioners.

The prescription patterns of anti hypertensive therapy at the Government Medical College, Chandigarh, revealed that 58.7% of the patients received a single drug while 29.7% were prescribed two or more drugs (Jhaj et al., 2001). β-blockers were the most popular choice accounting for 46.7%, followed by calcium channel blocker (34.3%) and angiotensin converting enzyme inhibitors (30%). The least used drugs were diuretics, which had a share of only 13.2%.

A cross-sectional study at a tertiary care hospital (Bangaluru), 65.9% ward patients were males and 34.1% females (Xavier et al., 2001). The mean age of males and females were 55.7 and 57.2 years, respectively. Calcium channel blockers were the most commonly prescribed drugs (28.1%), followed by angiotensin converting enzyme inhibitors (24.7%), β-blockers (22.6%), and diuretics 21.7%.

Walley et al (2003) found diuretics or beta blockers to be the most widely prescribed first-line treatments (54%) of hypertension in the UK between the period of January 1993 and December 1997.

At the Punjab University Health Center, 57.8% of the male patients were prescribed monotherapy and 42.2% combination therapy. Calcium channel blockers were the most prescribed agents (48.1%) and the diuretics the least prescribed (1.9%)
due to their adverse effects on glucose homeostasis and lipid profile (Tiwari et al., 2004).

The prescription patterns for hypertension in Taiwan indicated that calcium antagonists were the most prescribed medication, similar to the US but different from Britain, Spain, Italy and Canada (Chou et al., 2004). In contrast, beta blockers were the most prescribed drugs in Germany, Finland, Hungary and Hong Kong. Choice of the medicine was made depending upon the side effects, age and sex factors.

Deshpande (2005) studied the general prescribing patterns at the Nagpur city hospital. About 55% were on monotherapy and 45% on combination therapy. Among monotherapy, β-blockers were the most popular choice.

Jun et al (2006) reported the changes in the prescribing methodology of antihypertensive therapy between 1993 and 2004 in the US. In 2001, prescription of diuretics was 39%, which increased to 53% in first quarter of 2003. However these increases did not sustain in 2004. Thiazides and β-blockers were both prescribed mostly between 1998 and 2004, when the use of calcium channel blockers and angiotensin-converting enzyme inhibitors declined significantly.

A study of 690 patients in the Isfahan, Najafabad and Arak states of Iran found that 670 patients under treatment of antihypertensive drug therapy, 527 had been treated with one drug type, (Khosravi et al., 2006). Among these, the single drug, beta-blockers were the most common choice (23%), and diuretics were the least prescribed (0.9%).

Joshi et al (2006) reported a prescribing pattern of calcium channel blockers 65%, β-blockers 52%, diuretics 28%, and angiotensin converting enzyme inhibitors 19%. Amlodipine was the most prescribed drug in both monotherapy and combination therapy in Kathmandu, which is in line with the recommendation of British hypertension guidelines.

Mpe et al (2007) saw no uniform pattern of specific drug prescription as initial therapy but mostly diuretics and calcium channel blockers were used as monotherapy
for black patients (South Africa) as β-blockers and ACE displayed poor response in them.

At primary health care centers in Bahrain, 62.9% patients were found on monotherapy and 37.1% on combination therapy. As much as 58.8% β-blockers, 14.2% Angiotensin converting enzyme inhibitors, 11% calcium blockers, and 8.1% diuretics were used for monotherapy. Significant age and gender related difference in prescribing pattern were seen (Jassim et al., 2001).

The prescription pattern of antihypertensive therapy varied by age and gender (Liu et al., 2008). Calcium channel blockers and beta blockers were the most frequently used medicine either alone or in combination. Prescriptions of diuretics were low in uncomplicated hypertension in Taiwanese patients.

2.8 Interaction pathway of multiple genes in RAAS

A number of genes (Figure 1) codifying RAS have been involved in the etiopathogeny of hypertension (Sandro et al., 2007). Given the pivotal role of the renin-angiotensin-aldosterone (RAS) system for long-term regulation of blood pressure and
volume, it was obvious to consider RAS components as candidate genes in hypertension.

RAS functions as an endocrine system. The renin gene is expressed primarily in the juxtaglomerular cells of the kidney, where renin is synthesized, stored, and released into the circulation. Prorenin is cleaved to form renin, which is stored in tissue granules until it is released in response to specific secretagogues. Secretion of rennin from the kidneys is controlled by several factors. The macula densa are a specialized group of distal convoluted tubular cells that act as chemoreceptors for sodium and chloride levels in the distal tubule.

Sodium retention increases blood volume, which is followed by an increase in blood pressure. This increase in blood pressure activates a negative feedback regulation of the juxtaglomerular cells in the kidney, which sense renal perfusion pressure and renin production are inhibited. Renin secretion is autonomically modulated via sympathetic innervation of the renal tubules and arterioles. Circulating renin catalyzes the angiotensinogen-to-angiotensin I conversion. The angiotensinogen gene is expressed in the liver, the site of AGT synthesis and release into the circulation. The angiotensin I (Ang I) generated by renin activity is a vasoinactive decapeptide. Conversion of angiotensin I to angiotensin II (Ang II) is the key reaction in the RAS pathway, generating the effector of the system, Ang II, a potent vasoconstrictor. The reaction is catalyzed by ACE, a zinc metallopeptidase widely distributed on the surface of endothelial and epithelial cells. The ACE gene is confined to chromosome 17q23, comprises 21 kb and consists of 26 exons (Crissan et al., 2000). Numerous reports have linked a common insertion-deletion (I/D) polymorphism in the ACE gene with a wide variety of diseases including hypertension (Rosskopfs et al., 2007).

RAAS also plays important roles in the regulation of water and sodium balance with another candidate locus that has been widely investigated is α-adducin (ADD1) gene. Adducin is a heterodimeric protein involved in the assembly of the spectrin–actin cytoskeleton (Hughes et al., 1995). It modulates actin polymerization, binds calmodulin, is phosphorylated by protein kinase C (PKC) and tyrosin kinase, and regulates cell signal transduction (Matsuoka et al., 1996). Alpha adducin and epithelial sodium channels are important for sodium reabsorption. Adducin regulates the function
of sodium-potassium pump. The gene encoding alpha adducin is located in chromosome 4 (Manunta et al., 1999). A guanine to thymine substitution in position 614 of the gene causes a glycine to tryptophane substitution in position 460 of the polypeptide chain of adducing molecule. The presence of this polymorphism is associated with increase sodium reabsorption and arterial hypertension.

Catecholamines endogenous or exogenous provide their affects via adrenergic receptors. The gene encoding for β-1 adrenergic receptors is located on chromosome 10 (Yang-Feng et al., 1990). An adenine to guanine substitution in position 145 of the gene causes a glycine to serine substitution in position 49 of the receptor molecule. Two common polymorphisms in the β-1 ADR are Gly389Arg and Ser49Gly (Leineweber et al., 2009). The Ser49Gly polymorphism is located in the N-terminal region of the receptor protein, and the 49Gly allele is associated with enhanced agonist-stimulated down-regulation (Rhatz et al., 2002). Mechanistically, this differential behaviour is attributable to an altered N-glycosylation leading to an increased resistance towards receptor degradation upon internalization in the 49Ser allele. Furthermore, the 49Gly variant is associated with an increased basal activity (constitutive activity), a finding, however, that depends on the degree of receptor expression. β-1ADR also mediate lipolysis and regulate the release of renin and by this activation of the renin-angiotensin-aldosterone system.

Mechanism is abnormally activated in many patients with essential hypertension, and at least three mechanisms have been offered: nephron heterogeneity, non modulation, and increased sympathetic drive. Nephron heterogeneity with unsuppressible rennin secretion and impaired natriuresis as cause of essential hypertension: Within the kidneys, there exists a functional and structural basis for the abnormal rennin secretion and impaired Na+ excretion that are characteristic of hypertensive states (Guyton et al., 1995). In non modulation system normal rennin and high rennin levels seen in nearly half of hypertensive patients due to defective feed-back regulation of the rennin-angiotensin system within the kidneys and the adrenal glands (Williams et al., 1991). These findings have been attributed to an abnormally regulated and rather fixed level of ACE. The hypothesis that there is an abnormally regulated, fixed local angiotensin II concentration in these modulators received support from the correction of both the
adrenal and renal defects after suppression of Angiotensin II by ACE-inhibitors. Non-modulation in the face of relatively high dietary sodium intake could explain the pathogenesis of sodium sensitive hypertension and provide a more targeted, rational therapy for correction.

The relevant message is that each gene variant discloses its own function only when measured in the appropriate conditions. In particular, the predictivity of the pharmacologic response to a drug that somehow interferes with the disease-favoring allele is independent from the demonstration that the culprit allele is significantly associated with the disease this is the reason why pharmacogenomics may provide a paramount contribution (Ferrari et al., 2000; Evans et al., 1999; Roses et al., 2000; McCarthy et al., 2000). In the last decades, the selective blockade of a given receptor or biochemical activity with an appropriate drug has greatly contributed to our understanding of the complexity of cellular biochemistry and body pathophysiology.

Even though the drug selectivity was often not especially high, thus weakening the scientific validity of the information, the use of different drugs with different selectivity was of great help. Along this line, the availability of ACE inhibitors provided a substantial contribution to the comprehension of the pathophysiology of this system.

Why has this contribution been much greater than that resulting from 10 yr of statistical genetics studies on the polymorphisms of RAS genes? Even if there were problems of drug selectivity, it is not possible to ignore this big discrepancy. As mentioned above, the blockade of a given molecular biochemical function with a drug is one of the most powerful tools for understanding the function of a gene and its contribution to the overall activity of a complex system. The selective antihypertensive activity of a drug in patients carrying a given genotype or a combination of them may indicate a common pathway between the drug and the genotype and highlights a given genetic hypothesis over others.

2.9 Pharmacogenomics journey

One of the most challenging areas of research in pharmacogenomics is to understand why individuals respond differently to drug therapy, both in terms of beneficial as well as adverse effects (Storm, 2000). Important factors in interpreting the variability in the outcome of drug therapy include the patient’s health profile, prognosis,
disease severity, quality of drug prescribing and dispensing, compliance with prescribed pharmacotherapy and the genetic profile of the patient. The concept of altered responses based on genetic differences is not new. Fredrich Vogel first used the term pharmacogenetics in 1959. Peter Goodfellow, a senior researcher in the field of pharmacogenomics, recently stated that for each drug, on an average 30% of treated patients show beneficial effects, 30% do not show beneficial effects, 10% experience side effects and 30% are non-compliant (Feenstra, 1999).

Pharmacogenomics focuses on the question as to what extent variability in genetic make-up is responsible for the observed difference in therapeutic efficacy, effectiveness and adverse reactions in patients.

2.9.1 Association studies of different polymorphism

2.9.1.1 Angiotensin converting enzyme (ACE)

The gene encoding ACE is located on chromosome 15. The ACE gene polymorphism is characterized by either insertion (I) or deletion (D) of a 287-base pair sequence in intron 16 (Soubrier et al., 1993). The three possible genotypes are: II, ID and DD. Individuals with the DD genotype have increased plasma ACE levels, while individuals with II genotype have lower ACE plasma levels (Rigat et al., 1990 and Tiret et al., 1992). The DD genotype has been associated with increased risk of myocardial infarction (Cambien et al., 1992 and Lindpaintner et al., 1995), greater left ventricular dilation after anterior myocardial infarction (Pinto et al., 1995), greater left ventricular hypertrophy in hypertensive patients (Schunkert et al., 1994), and reduced survival in patients with heart failure (Andersson, 1996).

The response to ACE inhibitors is also affected by ACE genotype. Therapy with captopril in post myocardial infarction patients blunted left ventricular dilation in DD genotype patients but not in ID or II (Pinto et al., 1995). Similarly, regression of left ventricular hypertrophy with enalapril in hypertensive patients was greater in those carrying the DD genotype compared to the other two genotypes (Penno et al., 1998; Sasaki et al., 1996 and Van et al., 1996)
ACE gene polymorphism was reported to be associated with essential hypertension in African American and Australian Caucasian populations but no association was found among Arabs (Asamoah et al., 1996).

Yoshida et al. (2000) suggested positive association of ACE gene with elderly hypertensive patients. The II homozygote was significantly higher in elderly patients than in controls. There was no such association in middle age group.

Pasha et al. (2002) investigated for the first time ethnic variations in the frequency of the ACE gene I/D polymorphism in a well defined ethnic population(s) of India. Out of five groups, only the Sikhs showed marginally higher frequency of DD homozygote over the II homozygote. The other ethnic groups studied were Jats, Dogras, Assamese and Kumaonese. The author suggested that results reflected the influence of genetic drift, as found in many other polymorphisms such as blood groups. However, Gupta et al. (2009) suggested that the observed association between the DD genotype and hypertension reported by Pasha et al. (2002) were due to smaller population size.

In a study from Bangladesh, a significant association was observed between ACE I/D polymorphism and hypertension (Morshed et al., 2002). The frequencies of the D and I allele among the hypertensive patients were 69.3% & 30.6% and among controls they were 45.7% and 54.2%, respectively. Statistical analysis showed that the DD genotype was significantly higher among hypertensive subjects than control subjects. The mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) was significantly associated with the DD genotype in men but not in women.

Agachan et al. (2003) established positive association of ACE gene polymorphism with essential hypertension in a Turkish population. The D allele was higher in the hypertensive group compared to the control group.

No significant association of the three genotypes with hypertension, age and body mass index was observed among men (Bhavani et al., 2004). In the hypertensive group, men with DD homozygote showed maximum frequency of family history (52.2%) compared to controls (46.7%). Mean systolic blood pressure levels increased with an increase in the number of D allele and mean diastolic blood pressure decreased.
with increase in the number of D alleles. This difference was greater in hypertensive men with DD genotypes as compared to hypertensive women with DD genotypes, suggesting greater risk for men with family history of hypertension.

Ismail et al. (2004) reported significant association of the ACE II genotype with young hypertensive subjects but found no such association with DD genotypes in the northern Pakistani population.

ACE polymorphism has positive association with coronary artery disease (CAD) patients in the north Indian population of Uttar Pradesh (Agarwal et al., 2004). The frequency of D and I in the control and patient samples did not differ significantly. But the frequency of DD and ID genotypes was slightly higher in hypertensive patients than the controls: 21.9% vs. 18.49% for DD and 54.79% vs. 52.05% for ID genotypes. On the other hand, the frequency of II genotype was slightly higher in controls than in the hypertensive patients.

Glavnik et al. (2007) did not find any association between DD genotype and essential hypertension in the Slovene population.

Randhawa et al. (2006) suggested that ACE I/D polymorphism was not associated with essential hypertension in the Punjabi population with respect to age, height and weight. The incidence of hypertension was greater in women than in men. Patients with II genotype had high diastolic blood pressure as compared to those with genotype DD.

Sipahi et al. (2006) reported no differences in the allele frequencies and genotype distributions of ACE polymorphism between the control and hypertension groups. The frequencies of the genotype II, ID and DD were 31.9, 36.2 and 31.9%, respectively in men and 17.8, 51.1 and 31.1%, respectively in women. The frequency of DD genotype in the female hypertensive group was significantly higher than the control group. This study showed positive association between the ACE DD genotype and the development of hypertension in the female population of Trakya but no such association in the male group.
Saab et al. (2007) reported the frequency of D allele to be 73.42% in the middle-eastern populations, which is consistent with other studies.

Polupanov et al. (2007) found D allele to be less frequent in the Kyrgyz population compared to other Asian populations. Patients with the DD genotype had higher level of ACE activity than those with the II genotype.

The distribution of ACE I/D genotypes in the Greek population were significantly different than the Koreans and the Japanese Sekerli et al. (2008). The frequency of D allele was higher in the Caucasians than in the Korean and the Japanese.

2.9.1.2 Alpha Adducin (ADD1) polymorphism

Adducin is a heterodimeric cytoskeleton protein and consists of an α-subunit (103 kD) and either a β (97 kD) or a γ-subunit (90 kD). Three human genes (ADD1, ADD2, and ADD3) that map to different chromosomes encode these subunits (Matsuoka et al., 2000). Adducin is highly conserved through the different species, thus suggesting a role in basic cellular functions. Adducin is a ubiquitously expressed cytoskeleton protein that is involved in the formation of actin-spectrin lattice, actin polymerization, and cell signal transduction including an effect on Na-K ATPase (Joshi et al., 1991 and Matsuoka et al., 1996).

In humans, two polymorphisms of the ADD1 gene lead to amino acid substitutions: Gly460Trp and Ser586Cys (Cusi et al., 1997). Other polymorphisms occurring in humans are ADD2 and ADD3. The first linkage and case-control studies have demonstrated an association of the ADD1 Trp allele with hypertension (Cusi et al., 1997). Among hypertensive patients, plasma rennin is lower in carriers of the ADD1 Trp allele than in wild-type homozygotes. Patients with low rennin hypertension have higher BP in the presence of mutated Û-adducin, and ADD1 Trp/Trp homozygotes experience the largest increase in BP (Bianchi et al., 2005; Grant et al., 2002). Furthermore, carriers of ADD1 Trp allele, compared with the Gly/Gly homozygotes, showed an increased proximal tubular reabsorption measured by lithium clearance (Manunta et al., 1999) and a larger increase of BP after a saline infusion (Manunta et al., 1998).
An association study on the Japanese population showed negative results (Kato et al., 1998). Pathophysiology role of \textit{ADD1} and ethnic variations both were not found to be involved in the pathogenesis of hypertension.

No evidence of a significant difference in the frequency of the \textit{ADD1} G460W polymorphism was found between Scottish hypertensive and normotensive populations (Clark et al., 2000). Similar results were found in the Italian and French populations (Glariosoo et al., 1999).

Association of \textit{ADD1} with essential hypertension was not found significant in the Japanese and Chinese populations (Ranade et al., 2000).

Yamagishi et al., (2004) found no significant association between \textit{ADD1} polymorphism and blood pressure in either sex. But significant association was found among men above 55 years of age in the Italian population.

A cross-sectional study of \textit{ADD1} gene polymorphism and essential hypertension in the Korean population showed no positive association (Shin et al., 2004). Frequency of the 460Trp allele was 59.4\% in normotensive and 61.1\% in hypertensive people. The frequency was similar in other Asian populations (42-56\% in Chinese and 52-66\% in Japanese) but it was low in the white population (Italy 18\%, France 20\%) (Juz-Zhang et al., 2003).

He et al., (2003) found no significant association of \textit{ADD1} with essential hypertension in the Shanghai population. The frequency of 460 Trp variant (45.9\% to 48.2\%) was similar to that among the Japanese (54\% to 60\%) but much higher than the Caucasians (13\% to 23\%).

Alioglu et al., (2010) reported no association between \textit{ADD1} gene polymorphism and non-dipper phenomenon in a group of patients with essential hypertension suggesting that \textit{ADD1} does not exert a direct effect on vascular tone.

Ramu et al (2010) reported a largest case-control study to evaluate the role of Gly460Trp polymorphism in susceptibility to hypertension in South Indian population. They failed to find any association between them.
2.9.1.3 β-Adrenergic receptors polymorphism

The sympathetic nervous system is important to blood pressure regulation through its effects on cardiac function, peripheral vascular resistance, rennin release, and renal sodium handling. These regulatory systems, including the sympathetic nervous system itself, are influenced by genetic variance (Grim et al., 1979; Miller et al., 1980). The sympathetic nervous system, as reflected by catecholamine values, has been implicated mechanistically in essential hypertension (Campese et al., 1982). Thus, genes influencing catecholamine production, or those influencing α-adrenoreceptor and β-1 adrenoreceptor function, are potential candidates for hypertension.

β-1 adrenoreceptor (β-1 ADR) is expressed in the heart, it mediates increase in heart rate and contractility in the kidney, it coordinate release of rennin and thereby activation of the renin-angiotensin-aldosterone system, and in adipocytes, it helps lipolysis (Brodde and Michel, 1999). β-2 ADR is abundantly expressed on bronchial and vascular smooth muscle cells, where it mediates bronchodilation and vasodilation (Guimaraes and Moura, 2001). It is also expressed in the heart and can mediate positive inotropic and chronotropic effects but to a lesser extent than β-1 ADR, and is found in glands, lymphocytes and hepatocytes.

The β-1 ADR is encoded by an intronless gene located on chromosome 10q24–26, consisting of a short 5′ untranslated region (UTR) of 86 bp, an open reading frame that encodes a protein of 477 amino acid residues and 3′ UTR of about 900 bp (Frielle et al., 1987). There are 2 major single nucleotide polymorphisms (SNP) in the human β-1 ADR coding region. At position 49 in the amino-terminus of the receptor, a serine (Ser) is substituted by a glycine (Gly) Börjesson et al., 2000; Maqbool et al., 1999.), and Gly being the minor allele. At position 389 in the proximal part of the carboxy-terminus (a region that is within a Gs-coupling domain of the β-1 ADR), an arginine (Arg) is substituted by a Gly (Maqbool et al., 1999; Mason et al., 1999 and Tesson et al., 1999), where Gly is the minor allele. Codon 49 and codon 389 polymorphisms are in linkage disequilibrium so that the diplotype Gly49Gly/Gly389Gly occurs very rarely,(Terra et al., 2005). Interestingly, the Gly389 allele was present in the first clone of the
β-1 ADR and was therefore, although being the minor allele, was considered to be the wild-type (WT) β-1 ADR for a long time (Frielle et al., 1987).

Three studies in a group of (147, 223 and 40) essential hypertensive patients, not treated with any antihypertensive therapy, did not find any genotype-dependent differences in the resting blood pressure and the heart rate in patients homozygous for the Arg389 or Gly389 β-1 ADR (Shaughnessy et al., 2000; Liu et al. 2006; Johnson et al., 2003).

A case control study in 292 hypertensive patients and 265 normotensives controls found no association of Ser49Gly β-1 ADR polymorphism with the risk of hypertension (Bengtsson et al., 2001). Another study (Filigheddu et al., 2004) done on 526 essential hypertensive patients and 192 normotensives subjects from North Sardinia found no significant difference in the prevalence of Arg389Gly and Ser49Gly β-1 ADR polymorphisms. On the other hand, a study on the Japanese population reported positive association in the males but not in females (Shioji et al., 2004). Prevalence of Gly389 β-1 ADR variant was significantly lower in hypertensive patients than in normotensives subjects, leading to the conclusion that people carrying 1 or 2 Arg389 alleles have a higher risk of hypertension.

Due to the crucial role of β-1 ADR in the regulation of cardiovascular system, several attempts have been made to study the prevalence of Arg389Gly or Ser49Gly β-1 ADR polymorphisms in hypertensive patients. But the results were inconsistent. Two studies (Brodde, 2008) reported the association of Arg389 β-1 ADR polymorphism with a higher risk of hypertension, while six studies found no such association.

In a South Indian population, Ser49Gly polymorphism was reported to influence the cardiovascular responses to treadmill test, whereas Arg389Gly polymorphism failed to show similar response (Mahesh Kumar et al., 2008). The effect of metoprolol on exercise induced changes in cardiac parameter was not influenced by Ser49Gly and Arg389Gly polymorphism.

Ramu et al (2009) reported that homozygous variant genotype Gly49Gly of the Ser49Gly polymorphism was higher in hypertensive patients compared with controls. Positive interaction was seen in hypertensive patients carrying Ser49Gly/Gly49Gly x
Arg389Gly/Gly389Gly genotype. Significant interaction was observed between Ser49Gly polymorphism and essential hypertension in a south Indian population.

Ramu et al (2010) studied the genotypic and allelic frequencies of Ser49Gly and Arg389Gly polymorphisms in healthy Tamilian population and reported the frequencies of the variant alleles Gly49 and Gly389 as 15.1 and 25.8, respectively. The frequencies of homozygous Ser49Ser genotype in their population were almost similar to that of Swedish and Japanese population. They observed a moderate linkage disequilibrium (LD) between Ser49Gly and Arg389Gly polymorphisms which were reported to have weak or strong LD in Swedish and African-American populations, respectively.

Ramu et al., (2011) reported the association of different RAS gene polymorphisms with hypertension in Tamilian population. Positive association was observed between ACE I/D polymorphism and hypertension, however they failed to find any association between AGT T207M, M268T and AGT1R A1166C gene polymorphisms and hypertension.

2.10 Polymorphism associated with anti hypertensive drug response

No single genetic variant has emerged from linkage or association analyses as consistently related to blood pressure level or diagnostic category in every sample and in all populations. Knowledge of the genes that influence the Pharmacodynamics determinants of cardiovascular drugs provide insights on the molecular mechanism of drug action and better understanding of the interindividual differences in response to different classes of cardiovascular drugs. Some studies were found on the potential gene-drug interaction between genetic polymorphisms and antihypertensive drugs.

The ACE I/D polymorphism have been shown to be associated with BP response to angiotensin receptor antagonists, ACE inhibitors and diuretics with inconsistent results. The influence of genetic polymorphisms regarding the renin angiotensin system (RAS) and its response to angiotensin converting enzyme inhibitors (ACE inhibitors) was studied. Some studies have demonstrated an association of better BP response to a thiazide diuretic, an angiotensin receptor antagonist and different ACE inhibitors to the ACE II genotype (Ohmichi et al., 1997, Haas et al., 1998, O'Toole et al., 1998, Kurland
et al., 2001, Sciarrone et al., 2003). While others associate with the DD genotype (Stavroulakis et al., 2000, Liu et al., 2003). One study has even suggested a gender-specific association of BP response to hydrochlorothiazide with the ACE I/D genotype (Schwartz et al., 2002). Additionally, there are many earlier studies showing no significant difference in BP response with different angiotensin receptor antagonists, ACE inhibitors or other antihypertensive drugs between the ACE I/D genotype groups (Hingorani et al., 1995, Dudley et al., 1996, Harrap et al., 2003, Yu et al., 2003, Redon et al., 2005, Schelleman et al., 2006a, Schellemann et al., 2006c, Filigheddu et al., 2008).

On the other hand, following ACE inhibitor therapy hypertension-induced left ventricular hypertrophy was less decreased in patients with insertion allele (I allele) genotype (Eldesoky et al., 2006).

In the GenHAT study almost 37, 000 hypertensive patients were randomized to chlorthalidone, amlodipine, lisinopril or to doxazosin treatment and followed up for 4 to 8 years (Arnett et al. 2005). In that large study, there was no association between ACE I/D genotype group and BP response to study drugs, or with the primary outcomes of the study.

ACE genotype and ACE activity were predictive of 48.1% and 38.1%, respectively, of the variation in the ACE inhibition response to enalaprilat. Thus, ACE genotype predicted the response to the ACE inhibitor independently of ACE activity. However, although ACE genotype was the most important single factor for showing that ACE activity has an important regulatory role in the RAS (Ueda et al., 1998)

Twelve subjects responded and/or normalized with ramipril once daily, where the office and 24-h ABP were decreased significantly from baseline (p < 0.01). The percentage and magnitude of 24-h SBP/DBP loads after treatment were significantly decreased from 92 + 9.7/91 + 15.9 to 67 + 23.8/65 + 27.6 (p < 0.01) and from 23 + 10.6/16 + 5.3 mmHg to 17 + 10.3/10 + 4.8 mmHg (p < 0.05). Ramipril 2.5 and 5 mg once daily exerted the smooth 24-hour blood pressure reduction in essential hypertensive Thai patients (Uchaipichat et al., 2008).

No significant association of angiotensin-converting enzyme gene polymorphism with essential hypertension was found. Angiotensin-converting enzyme
gene polymorphism might be related to the antihypertensive response to an angiotensin-converting enzyme inhibitor in hypertensive patients (Xiaotao et al., 2003).

ACE polymorphism correlates with plasma ACE activity which is higher in those with the deletion (DD) allele (Dudley et al., 1996, Dieguez-Lucena et al., 1996). In one parallel study in patients with essential hypertension the ACE polymorphism was determined before 15 days of treatment with either an ACE inhibitor (enalapril), a calcium antagonist (verapamil) or a β-blocker (bisoprolol). The ACE polymorphism was associated with response to antihypertensive therapy, where enalapril produced a greater reduction in blood pressure in the DD variant and verapamil produced a more consistent decrease in blood pressure in the II variant. In contrast, 2 larger studies found no association between ACE genotype and response to antihypertensive therapy (Dudley et al., 1996, Hingorani et al., 1995). Similarly, in a Japanese study there was no difference between ACE genotype in blood pressure response after treatment with enalapril for 1 year (n = 60).

Some family studies showed associations between adducin alleles and blood-pressure parameters or demonstrated linkage with the α-adducin locus, while others failed to do so (Cusi et al., 1997). Similarly, the majority of studies addressing blood pressure in general, predominantly normotensive populations failed to show associations with adducin polymorphisms, reported positive associations for subgroups only (e.g. postmenopausal women), or depended on epistatic interactions with other polymorphisms, especially the ACE I/D polymorphism.

The results reported by Suonsyrjä et al., 2008 showed no effect for the ACE I/D polymorphism on BP response to losartan, amlodipine, bisoprolol or hydrochlorothiazide.

One of the study reported to compared with the baseline systolic BP (SBP) of subjects with one ACE I allele and one ADD1 Trp allele, the baseline SBP of those with ACE DD and ADD1 Gly/Gly genotypes was significantly higher. However, no associations were found between the interaction of ACE I/D and ADD1 Gly460Trp polymorphisms and the baseline diastolic BP or the BP response to Benazepril treatment. Our results suggested that the interaction effect of ADD1 Gly460Trp and
ACE I/D polymorphisms might play a significant role in regulating baseline BP but not BP response to Benazepril in Chinese population (Yunxian et al., 2005).

The relation between salt-sensitive hypertension and I/D ACE gene polymorphism has been previously tested in several studies with controversial results. (Kojima et al., 1994) reported a lack of association between the ACE I/D genotype and salt sensitivity in patients with essential hypertension, but they observed that PRA increase after salt restriction is greater in patients with the DD genotype. (Hiraga et al., 1996) instead reported a significant association between salt sensitivity and ACE genotype that has been confirmed by (Giner et al., 2000) who found that II patients are the most responsive to acute changes in sodium intake.

In a subsequent study it was found that the α-adducin locus was associated with hypertension (Casari et al., 1995; Cusi et al., 1997) reported that the Gly460Trp polymorphism in the human ADD1 gene was associated with hypertension, noting that hypertensive patients heterozygous for the 460Trp allele had better BP response to two month treatment with hydrochlorothiazide compared to wild-type homozygotes (14.7 vs. 6.8 mmHg). The mechanism for these findings has been suggested to be that the ADD1 460Trp allele is linked to higher activity of the sodium pump, thereby leading to increased tubular reabsorption of sodium in the kidneys, and ultimately to salt-sensitivity and hypertension (Manunta et al., 1998; Ferrandi et al., 1999; Manunta et al., 1999). Consistent with these findings, it has also been reported that hypertensive patients carrying the Trp allele have a larger BP increase in response to saline infusion and lower PRA compared to the GlyGly homozygotes (Cusi et al., 1997; Glorioso et al., 1999; Barlassina et al., 2000b).

In two prospective population studies, the 460Trp allele was associated with increased risk of total and cardiovascular mortality, as well as to cardiovascular, cardiac and coronary events (Gerhard et al., 2008). In a third prospective study, the 460Trp allele was associated with increased risk to ischemic and hemorrhagic stroke in blacks (Van Rijn et al., 2006). In addition to the study of Cusi et al., 1997, the 460Trp allele has been associated with better BP response to hydrochlorothiazide in three other studies with newly diagnosed hypertensive Italian subjects. Glorioso et al., 1999 performed a prospective study with 143 hypertensive patients from Milan and Sassari.
and confirmed that BP response to two months of hydrochlorothiazide treatment was better in patients carrying the 460Trp allele. Correspondingly, Sciarrone et al., 2003 demonstrated that hypertensive patients carrying at least one ACE I allele and one ADD1 Trp allele had the best mean BP response to hydrochlorothiazide (12.7 mmHg vs. 3.4 mmHg in DD-GlyGly group). In the most recent study using 193 hypertensive Italian subjects, both systolic and diastolic BP response to one-month treatment of hydrochlorothiazide was significantly better in subjects with the 460Trp allele (Manunta et al., 2008). None of these four studies with positive results have been placebo-controlled. There is a population-based case-control study showing that in subjects carrying the ADD1 460Trp allele, diuretic therapy was associated with a lower risk of combined myocardial infarction and stroke compared to other antihypertensive therapies (Psaty et al., 2002).

Despite the promising results on the association of the ADD1 460Trp allele with enhanced BP response to hydrochlorothiazide in four Italian studies, the same association has not been reproduced in studies using other populations. Turner et al. could not demonstrate any Gly460Trp polymorphism-related effect on BP response to four week treatment with hydrochlorothiazide, in a study of 291 African Americans and 294 non-Hispanic whites (Turner et al., 2003). Negative results were also obtained in two cohort studies from the Netherlands (Schelleman et al., 2006a, Schelleman et al., 2006b). Supporting these results, in the GenHAT study, with a total of 37,000 individuals, there was neither association of the Gly460Trp genotype with BP response to chlorthalidone, amlodipine, lisinopril or doxazosin, nor better primary clinical outcome in subjects carrying the Trp allele whilst on chlorthalidone treatment (Davis et al., 2007).

The study does not support the assumption that the 460Trp allele is associated with stronger BP response to diuretics or other antihypertensive drugs. In fact, there was even an opposing trend, as the 460Trp allele was associated with a decreased BP response to hydrochlorothiazide. Collectively, the present study along with previously published studies suggests that the ADD1 460Trp allele is not a useful clinical marker of enhanced BP response to thiazide diuretics (Suonsyrjä et al., 2008).
In a prospective study of 40 hypertensive patients, Johnson et al. 2003 found that 24-hour and day-time diastolic ABP responses to metoprolol were greater in patients homozygous for the Arg389 allele compared to carriers of the Gly389 allele (-12% for Arg389Arg vs. -5.1% for Gly389 carriers). They also discovered that patients with the haplotype Ser49Ser/Arg389Arg had significantly better diastolic ABP response than patients with the haplotype Ser49Gly/Arg389Gly. In that small study, there was a marked racial imbalance between the genotype groups, and the metoprolol doses were varying since they were titrated according to BP responses. However, these findings are supported by a study with 61 Chinese hypertensive patients. This study showed that the best response to four week treatment with metoprolol was in patients homozygous for the Arg389 allele and in patients with the haplotype pair Ser49Arg389/Ser49Arg389, respectively (Liu et al., 2006). They also found that systolic BP response to metoprolol was better in patients homozygous for the Ser49 allele, compared with Ser49Gly heterozygotes. However, subjects with the haplotype pairs Ser49Arg389/Ser49Gly389 and Gly49Arg389/Gly49Arg389 of β-1 ADR were excluded from the analyses, with the study population being selected from a total of 223 previously genotyped patients. In addition, two other small studies with healthy volunteers have noted similar results (Liu et al., 2003; Sofowora et al., 2003).

The positive findings of association between the Arg389 allele and increased BP response to beta-blockers (Johnson et al., 2003; Liu et al., 2006) have brought high expectations upon Arg389 as a clinically relevant tool for treatment of hypertension by some of the authors (Shin et al., 2007). In one of the review articles of the field, the reports of Johnson et al., 2003 and Liu et al., 2006 have been seen as key articles to cardiovascular pharmacogenomics (Aquilante et al., 2009). However, three other studies with 52-270 hypertensive patients, and one study with healthy volunteers, failed to demonstrate any difference in BP response to atenolol or metoprolol between the Ser49Gly and Arg389Gly genotype groups (O'Shaughnessy et al., 2000; Filigheddu et al., 2004; Karlsson et al., 2004; Kurnik et al., 2008). Thus each analysis approach indicated that the Arg389Arg genotype was an important predictor of DBP lowering with metoprolol.
Considering the available data as a whole, one may conclude that the Arg389Gly polymorphism is not associated with variation of BP response to beta-blockers. In the present study ABP response to bisoprolol was slightly better in \( \beta-1 \) ADR Ser49Ser homozygotes compared to Ser49Gly heterozygotes. However, the significance of this association remains obscure (Suonsyrjä et al., 2008).

### 2.11 Rationale for the selected candidate gene polymorphisms

In the present study, the tested polymorphisms were chosen on the basis of functional relevance, either to BP regulation or through reported association with hypertension or antihypertensive drug response.

The survey of literature shows that there is very little data on the prevalence of hypertension in rural population of Haryana. Moreover, no pharmacogenomics study has been undertaken in rural population of Haryana till date for studying the association of gene polymorphism with severity of hypertension or variability of drug response among patients.

This study is undertaken to add useful information to improve patient care through advanced identification of patients at risk for essential hypertension and initiate slips to achieve personalized pharmacological treatment wherever possible.