Human essential or primary hypertension (EH), as a genetic disorder is widely debated in the past few decades. Wilhelm Weitz in 1923 produced first evidence for the fact that blood pressure might be heritable. He observed that 77% of parents of hypertensive patients suffered stroke and other heart diseases; whereas only 30% of parents of normotensives had these diseases (Chern and Chiang, 2004). In close view with this concept, Sir George Pickering and Sir Robert Platt initiated the path in the field of hypertension Genetics. About five decades ago, Sir George Pickering hypothesized that as the determinants of blood pressure were many, attributing hypertension to a single cause would be complex. On the other hand, Sir Robert Platt proposed that hypertension had a single discreet identifiable cause, which is true when considering the Mendelian forms of HTN (Thibonnier and Schork, 1995). Although their ideas were divergent the underlying quest for discovering genes associated with HTN has laid the foundation for the contemporary research in this field of study.

Essential hypertension (EH) is defined as a chronic increase in blood pressure (BP) for which there is no known, single underlying cause. BP must be tightly regulated to ensure uninterrupted perfusion to all vital organs. A transient interruption in the blood flow leads to loss of consciousness. Conversely, high BP provides no metabolic gain but might increase damage to blood vessels and vital organs (Lifton et al., 2001). EH is the most important modifiable risk factor for coronary artery disease, stroke, congestive heart failure, end-stage renal disease and peripheral vascular disease (Ettner et al., 2012). The renal, neural and endocrine systems regulate blood pressure by an intricate network of
physiological pathways involving extracellular fluid volume homeostasis, cardiac contractility and vascular tone. Any derangement in these pathways due to intrinsic (genetic) or extrinsic (environmental) factors or a combination of both can result in low or high blood pressure (Padmanaban et al., 2012).

2.1 EPIDEMIOLOGY OF ESSENTIAL HYPERTENSION:

Essential hypertension has evolved as a global challenge afflicting one billion people worldwide (~ 4.5% of the world population), accounting for 9.4 million deaths per year (Lim et al., 2012). Due to increasing longevity in the past few decades, the prevalence of EH is shown to rise with age, as it affects 25 - 35% of the adult population and about 60 – 70% beyond 70 years (Staessen et al., 2003). Blood pressures also change patterns, as age advances. Systolic BP increases with age and continues throughout life in contrast to DBP. DBP is a potent cardiovascular risk factor until 50 years of age, whereas SBP is associated with the most common form of HTN above 50 years (Franklin et al., 2001). The prevalence is also higher in men than women before 60 years of age, but equal after this age. Both men and women who were normotensive at age 55 - 65 and survive upto 80 – 85 years have a life time hypertension risk of 90% (Vasan et al., 2002). Recent epidemiological studies formed the basis of seventh report of the Joint National Committee (JNC 7) on prevention, detection, evaluation and treatment of high blood pressure has proposed a revised classification for hypertension that is shown in figure 1 (Chobanian et al., 2003).
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Figure 1. Classification of blood pressure (JNC 7).

2.1.1 Global scenario:

Several studies have reported the prevalence of hypertension in various regions of the world. The prevalence of hypertension in rural Chinese population studied by Dong et al. (2008), reported 37.8% of the age group 35 – 85 years had hypertension. Overall prevalence was higher in women (38.6%) than in men (37.0%). Age adjusted prevalence of hypertension based on Joint National Committee 7 criteria in Korean population was 22.9% (26.9% in men and 20.5% in women) (Choi et al., 2006).

Wolf-Maeir et al. (2003) reported the HTN prevalence among 35 – 64 years old individuals in six European and two North American countries. According to the report, the prevalence was highest in Germany (55%), Finland (49%), Spain (47%), England (42%), Sweden (38%), Italy (38%), United States (28%) and Canada (27%). Whitfield et al. (2009) have reported HTN as a major cause of death and morbidity among African-Americans. More than 50% of the African-Americans have been diagnosed with HTN by the age of 51 – 61 years.
Epidemiological studies have indicated strong associations with socioeconomic status, body mass and smoking among African-Americans and Caucasians.

Developed nations with more ageing population are presumed to have a higher prevalence of HTN than developing nations with younger population. Lack of awareness, lifestyle modification, influence of comorbid diseases such as diabetes and non-compliance with treatment are the confounding factors underlying such huge rising statistics of this modern epidemic. Prompt diagnosis and treatment could reduce the global burden of the disease.

2.1.2 Indian scenario:

The prevalence of EH has been increasing in India, both in rural and urban areas. Pooled epidemiological data obtained from various parts of India have reported that hypertension has increased by 30 times over a period of 55 years in urban population and 10 times over a period of 36 years in rural population (Gupta, 1997). A community based cross-sectional study in rural Maharashtra including 1297 subjects aged 19 years and above reported a hypertension prevalence of 7.24% (94 subjects). Of the 94 hypertensive subjects examined, the lowest prevalence of HTN was reported in individuals of age group 19 – 28 years and highest prevalence of 31% was recorded among individuals of age group >79 yrs. In males, prevalence of hypertension was less (44.68%) as compared to females (55.22%) (Todkar et al., 2009). A recent study by Gupta et al. (2011), reported an overall HTN prevalence of 38.2% in a rural population of Haryana.
A cross-sectional study of the urban industrial population across 10 sites in India by Reddy et al. (2006) revealed highest prevalence of HTN among men (49.2%) and women (62%) of age group ≥ 60 yrs. Gupta et al. (2004) performed a population based epidemiological study in the city of Mumbai. The study population included 88,653 subjects of which 48% were hypertensive. Interestingly, the prevalence of HTN was recorded marginally higher in the case of female (48.4%) than in male (47.5%) subjects.

Kalavathy et al. (2004), reported a prevalence rate of 51.8% in community dwelling elderly subjects falling in the mean age group of 70 yrs in Kerala. An epidemiological study by Mohan et al. (2007), on 2,350 subjects under phase 3 of Chennai Urban Rural Epidemiology Study (CURES-52) among individuals of age group ≥ 20 yrs to evaluate the prevalence, awareness and control of hypertension in Chennai. As per the report, 20% of the study population was found to be hypertensive. Of the participants within the age group of 20 – 29 years, 3.8% of men and 3.1% of women were hypertensive, while in subjects older than 60 years 50.8% of men and 51% of women were hypertensive. The overall prevalence of isolated systolic (SBP ≥ 140 and DBP < 90 mmHg) hypertension and diastolic (DBP ≥ 90 and SBP < 140 mm Hg) hypertension was found to be 6.6% and 4.2% respectively. The elderly population of age 60 years and above had 25.2% of isolated systolic hypertension which is due to stiffening of large arteries. The data provided clearly shows that escalation in blood pressure is associated with age. The effect of environmental influences on BP cannot be underestimated because a 10-kg rise in body weight is associated with
3mm Hg rise in systolic BP and 2.2 mm Hg rise in diastolic BP (Dyer and Elliott, 1989). Similarly, a decrease in daily sodium intake of 100mmol/day lowers systolic BP by 1 mm Hg and diastolic BP by 0.1 mm Hg (Midgley et al., 1996). It is also speculated that changes related to traditional dietary habits, lifestyle patterns and longer life expectancy predispose Indians living in urban areas more susceptible to hypertension.

2.2 GENETIC BASIS OF HYPERTENSION:

EH being a complex, quantitative trait is diverse across individuals. Ethnic and genetic heterogeneity influences variable clinical presentation and drug response in individuals making genetic study on hypertension to be a challenging task (Shih and O’Connor, 2008). The heritability of essential hypertension as suggested by twin and family studies is in the range of ~50% (Kupper et al., 2006). Relative risk of hypertension when a sibling is affected the sibling’s recurrence risk has been estimated as 2.5 to 3.5 (Tobin et al., 2007). The variations in blood pressure that are genetically determined are termed as “inherited BP”. Several other factors such as obesity, insulin resistance, smoking, high alcohol intake, high salt intake, low potassium and calcium intake, sedentary life-style, aging and stress, denoted as “hypertensinogenic” factors are known to elevate blood pressure. While, inherited blood pressure is considered to be the core BP, these hypertensinogenic factors tend to increase BP over and above the range of inherited BP (Carretero and Oparil, 2000).
Cardiac output and peripheral resistance are two major phenotypes which are found to operate together and regulate blood pressure. Cardiac output is the pressure required to move blood through the circulatory network which is provided by the pumping action of heart and peripheral resistance refers to the tone of arteries. These major phenotypes are in turn controlled by intermediate phenotypes including autonomic nervous system, vasopressor and vasodepressor hormones, structure of cardiovascular system, blood fluid volume and renal function. Hence, there could be a network of several genes participating in the development of hypertension (Vikrant and Tiwari, 2001). A wide range of studies encompassing various thrust areas have been designed to unravel the genetic basis of HTN.

2.2.1 Mendelian disorders: Studies on rare Mendelian forms of hypertension and hypotension have revolutionized our knowledge of blood pressure regulation. Two extensively studied examples of hypertension caused by monogenic mutations inherited in an autosomal dominant pattern are Liddle’s syndrome and glucocorticoid remediable aldosteronism (GRA). The former is due to mutation at the 16p13-12 locus coding for epithelial sodium channel which leads to an alteration or deletion in the cytoplasmic tails of β or γ subunits. The latter is the result of chimeric gene formed by the fusion of promoter-regulatory region of 11-β-hydroxylase and structural portion of aldosterone synthase which leads to the production of abnormally high levels of aldosterone synthase leading to elevated blood pressure. Several other disorders such as
Gordon’s syndrome, brachydactyly type E, Bartter’s syndrome, Gitelman’s syndrome were subsequently identified and has provided key evidence for the association of genes in the regulation of blood pressure (Luft, 2003).

2.2.2 **Twin studies:** The effects of genetic variance on a quantitative trait like EH can be clearly elucidated by twin studies. Monozygotic twins (MZ) share all genes in common and dizygotic twins (DZ) share on an average “half” their genes. Environmental confounders are minimized in twin studies since they share similar environments. Hypertension is about twice as common in individuals who have one or two hypertensive parents. Blood pressure is more closely correlated in MZ twins than DZ twins (Luft, 2001). The heritability data for BP from more than 4000 twin pairs from six different countries reveal 52 – 66% for SBP and 44 – 66% for DBP. With such an enormous number of twin pair studies one can confidently assert that more than 50% of variance in BP is ascribed to genetic factors (Evans *et al.*, 2003).

2.2.3 **Linkage studies:** Linkage analysis is used to map genetic loci in related individuals. Alleles of two loci which are sufficiently close to each other tend to cosegregate within families. Two loci are considered to be linked if they are transmitted from parent to offspring more often than expected. Thus, linkage analysis can be used to identify large genomic regions that contain disease predisposing genes. Krushkal *et al.* (1999) provided the results of first genome wide linkage analysis to identify genes that influence interindividual BP
variations in humans. Several regions that are likely to contribute to variations in SBP levels identified viz., 2p22.1-2p21, 5q33.3-5q34, 6q23.1-q24.1 and 15q25.1-15q26.1 are known to be significantly linked to genes that influence interindividual SBP variations. These regions contain known genes that have been suggested to affect blood pressure regulation at physiological levels. One such example is the product of the aminopeptidase N gene on chromosome 15 which regulates vasopressin release via the mechanism of the N-terminal cleavage of angiotensin III (Zini et al., 1996). In contrast to the above findings, the renin gene on chromosome 1 did not generate any significant linkage pattern with SBP. Since EH is likely to have low or medium penetrance, the susceptibility variants might not be subjected to strong selection as other monogenic disorders. This results in lower allelic heterogeneity and greater prevalence of the trait leading to inconsistent results. Hence, linkage analysis has not been very powerful as other methods in dissecting out the genetic basis of HTN (Risch and Merikangas, 1996).

2.2.4 Association studies: As with complex disorders, the focus has been moved from linkage studies to association studies in recent years, due to its greatest power to detect variants even with modest effects. Association studies have been facilitated by human genome sequencing and discovery of SNPs, such as International HapMap project (Frazer et al., 2007). Two approaches of association studies are: (i) indirect (gene-wide) candidate gene study and (ii)
genome wide association study. These approaches follow a case-control pattern wherein a gene-disease association is established.

2.2.4.1 Candidate gene studies: Hypothesis based candidate gene studies have discovered more than 100 genes found to be associated with BP. Traditionally, these tests are based on prior information about the genes involved in the physiological pathways involved in a disease phenotype. The genetic components of renin angiotensin-aldosterone system, the epithelial sodium channel, adrenoceptors, G-protein subunits are all classic examples of candidate genes that have been extensively studied so far in various populations. Analysis of these loci explain only a very small portion of the heritability of BP; indicating that many more common variants, or an extremely large number of rare variants, still remains to be discovered (Rana et al., 2007).

2.2.4.2 Genome-wide association studies (GWAS): Hypertension is one of the first complex traits studied under GWAS. The Wellcome Trust Case Control Consortium performed the first large collaborative GWAS for hypertension. This study did not produce significant association with the SNPs selected. Since then about eight individual GWAS for BP and HTN have been carried out globally providing information about novel variants with subtle effect on BP. Significant association was achieved in a meta-analysis performed by Wang et al. (2009) in an Amish population. The study identified a cluster of SNPs on chromosome 2q24.3 within STK39 gene coding for serine threonine
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kinase, which is known to interact with cation transporters in the nephron. Org et al. (2009) in a European study, showed an SNP upstream of CDH13 gene associated with diastolic BP. An intergenic SNP near the ATP2B1 gene, known to be involved in calcium homeostasis has been described by Cho et al. (2009). The Global BPGen Consortium and the Cohorts for Heart and Ageing Research in Genome Epidemiology BP Consortium, meta-analyzed GWAS data from 34,433 and 29,136 individuals, respectively. Both consortia identified genome-wide significant associations at 8 loci, with 3 of these loci being common to both studies (Levy et al., 2009; Newton-Cheh et al., 2009).

Although a wide array of susceptible loci for hypertension has been mapped through various studies, their association is found to affect only a very small proportion of the total variation in SBP or DBP (~0.05–0.10%, approximately 1 mm Hg per allele SBP or 0.5 mm Hg per allele DBP) individually (Newton-Cheh et al., 2009). But, the conjoint effect of such multiple risk alleles on blood pressure levels amount to rise in several mm Hg, which is sufficient to increase cardiovascular disease risk. Observational data indicate that a prolonged increase in DBP of 5 mm Hg is associated with a 34% increase in risk for stroke and a 21% increase in risk of coronary events (Levy et al., 2009). Linkage analysis, candidate gene approach, genome-wide association studies and others have contributed immensely to genetic research on hypertension. The substantial progress over the past few decades has led to detailed characterization of new BP loci which will provide targets for development of newer therapies for preventing and treating hypertension.
2.2.5 Animal models: Genetic animal models for HTN started with rat models which develop high BP spontaneously without any pharmacological or surgical intervention. Sibling mating of these hypertensive rats strains over several generations was adopted to raise spontaneously hypertensive rats (SHR) (Okamoto and Aoki, 1963). SHR and stroke-prone SHR develop HTN and target organ damage similar to human essential hypertension (Okamoto et al., 1986). Several inbred rat models viz., New Zealand genetically hypertensive rats (Smirk and Hall, 1958), Sabra hypertensive rat (Ben-Ishay et al., 1972), Lyon hypertensive rat (Sassard et al., 2003), Fawn hooded hypertensive rat (Kuijpers and Gruys, 1984), obesity-prone Sprague-Dawley rats (Dobrian et al., 2000), obese Zucker (Alonso-Galicia et al., 1996) and Wistar fatty rats (Yamakawa et al., 1995) are available for studying the physiological basis of hypertension. Apart from aforesaid inbred strains, genetically engineered animal models have also contributed in dissecting the molecular basis of HTN (Sarikonda et al., 2009).

Functional analysis is often studied by either overexpression or deletion of crucial genes of known physiological systems such as renin angiotensin-aldosterone system involved in the pathogenesis of HTN. TGR (mREN2)27, a transgenic rat strain, was obtained after incorporation of the murine renin gene which resulted in hypertensive rats (Mullins et al., 1990). Transgenic mice strains with activated human renin and angiotensinogen genes also resulted in elevation of BP (Yang and Sigmund, 1998). Gene knockout models have been constructed to determine the significance of the gene, by comparing
heterozygous (+/-) and homozygous (-/-) knockout phenotypes. Some examples of gene knockout mice models are atrial natriuretic peptide knockout mice that have salt-sensitive HTN (John et al., 1995) and endothelial nitric oxide synthase knockout mice that are hypertensive (Sheseley et al., 1996). Furthermore, consomic strains (chromosome substitution) (Cowley et al., 2004) and congenic strains (QTL substitution) (Rapp and Deng, 1995) have been developed to study etiology of EH in a more comprehensive manner.

2.3 CANDIDATE GENES UNDER STUDY:

2.3.1 Plasma membrane calcium ATPase (ATP2B1):

Calcium signaling is a dynamic process which regulates a variety of important cellular functions such as secretion, contraction and gene transcription. Ca\(^{2+}\) homeostasis is strictly controlled by molecular players which mediate the uptake and reflux of extracellular and intracellular Ca\(^{2+}\) ions (Berridge, 2001). SPCAs (secretory-pathway calcium ATPases) encoded by ATP2C1-2 genes, SERCAs (sarcoplasmic reticulum/endoplasmic reticulum calcium ATPases) coded by ATP2A1-3 genes and PMCAs (Plasma membrane calcium ATPases) encoded by ATP2B1-4 genes are specialized Ca\(^{2+}\) pumps which can take up intracellular calcium (Strehler and Treiman, 2004). In mammals, the PMCAs are the products of four distinct genes (ATP2B1-4), located on human chromosomal loci 12q21-q23, 3p25-p26, Xq28 and 1q25-q32 respectively (Olson et al., 1991; Latif et al., 1993). Although these pumps exhibit both structural and functional
differences, all three belongs to a group of P-type (phosphorylation) family (Leva et al., 2008).

![Figure 2. Location of ATP2B1 gene on chromosome 12 and its anatomy](http://genome.ucsc.edu/)

The PMCA pump was discovered by H.J. Schatzmann in the year 1966 from the red cells (Schatzmann, 1966). The PMCA1 gene contains 22 exons and a putative alternative exon 1* (Hilfiker et al., 1993). But recent report from database presents 21 exons in the ATP2B1 gene (http://asia.ensembl.org/) (Figure 2). Alternative splicing of PMCA gene generates upto 30 isoforms of PMCA gene products. PMCA isoforms show developmental, tissue and cell type-specific patterns of expression. PMCA1 and PMCA4 are expressed in all organs,
tissues and cell types (Dalley et al., 2006). Isoforms PMCA2 and PMCA3 are normally up-regulated in neuronal development (Usachev et al., 2001). The loss or malfunction of specific Ca\textsuperscript{2+} pump isoforms is associated with defects such as deafness, ataxia or heart failure (Strehler and Treiman, 2004).

The basic function of PMCA is to maintain ~10000 fold Ca\textsuperscript{2+} gradient across the plasma membrane by active extrusion of Ca\textsuperscript{2+} from the cell (Shull, 2000). PMCA pump transports one Ca\textsuperscript{2+} ion per molecule of ATP hydrolyzed. This energy expenditure is necessary to maintain a relatively low intracellular concentration of Ca\textsuperscript{2+}. Unstimulated PMCA pumps have poor affinity for Ca\textsuperscript{2+} and would be inactive at physiological sub µM cytoplasmic Ca\textsuperscript{2+} concentrations.

Calmodulin serves as an activator and regulator of PMCA pump (Jarrett and Penniston, 1977). Ca\textsuperscript{2+} influx across the plasma membrane or release from intracellular organelles are required for excitation–contraction coupling and receptor-mediated Ca\textsuperscript{2+} signaling (Hussain and Inesi, 1999). A high concentration of intracellular calcium causes endothelial cells to contract, constricting the blood vessel and reducing blood flow. Incidentally, calcium channel blockers are frequently prescribed to lower blood pressure.

2.3.1.1 Calcium ATPase and hypertension:

In vascular smooth muscle cells, altered function in Ca\textsuperscript{2+} channels increases contractility which is known to be a vital process in the development of essential hypertension. Increased Ca\textsuperscript{2+} influx into VSMCs (vascular smooth
muscle cells) may contribute to increased contractility and promote rise in blood pressure. Studies on animal models have revealed that $\text{Ca}^{2+}$ influx is greater in VSMCs from spontaneously hypertensive rats as compared to normotensive Wistar-Kyoto control rats (van Breemen et al., 1987).

Touyz et al. (1992), investigated levels of total calcium, magnesium, potassium and sodium in erythrocytes and platelets of hypertensive patients. The outcome of the study revealed that the platelet sodium, calcium, erythrocyte calcium were elevated while serum potassium, serum magnesium and platelet magnesium were decreased. These results suggested that intracellular sodium and calcium overload and magnesium depletion may be important in the pathophysiology of hypertension.

Similar study performed by Fu et al. (1998), assessed the relationships between plasma and intracellular $\text{Ca}^{2+}$, $\text{Mg}^{2+}$ and blood cell membrane adenosine triphosphatase (ATPase) activity by colorimetric analysis in normotensive and hypertensive subjects. The results showed a significant decrease in the activity of ATPase among the hypertensive group, with significantly lower plasma $\text{Ca}^{2+}$ and higher cytosolic $\text{Ca}^{2+}$ levels when compared to normotensive group. Plasma $\text{Ca}^{2+}$ depletion and cytosolic $\text{Ca}^{2+}$ overload can be attributed to defective transport mechanisms.

Large-scale genome wide association studies by the CHARGE, Global BPgen consortium and other candidate gene studies in European (Levy et al., 2009), Korean (Hong et al., 2010a, Hong et al., 2010b), East Asian (Xi et al., 2012) and Japanese (Takeuchi et al., 2010) population have identified $\text{ATP2B1}$
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as a hypertension susceptibility gene. The variants of this gene have been linked to all three BP traits including systolic BP, diastolic BP and hypertension. Furthermore, many functional analyses have also been carried out to elucidate the role of \( ATP2B1 \).

Recently, the functional role of \( ATP2B1 \) in blood pressure control has been proved in an in vivo model recently. A conditional knockout (KO) mouse model of \( ATP2B1 \) employing the Cre-loxP system specific for VSMCs was constructed to reveal the function of the gene. This study demonstrated that BP was significantly higher in mice lacking \( ATP2B1 \) in VSMCs compared to wild type mice. Inhibition of \( ATP2B1 \) caused three fold increment in intracellular calcium ion concentration. In addition, alteration in calcium ion homeostasis in VSMCs and increased vasoconstriction of femoral artery were observed in \( ATP2B1 \) KO mice. These results suggest that \( ATP2B1 \) has important roles in maintaining calcium ion contraction in VSMCs (Kobayashi et al., 2012).

2.3.2 Pleckstrin homology domain, family A, member 7 (PLEKHA7):

\( PLEKHA7 \) was discovered as a novel protein component of epithelial adherens junctions (Meng et al., 2008; Pulimeno et al., 2010; Pulimeno et al., 2011). The location of \( PLEKHA7 \) gene and its anatomy is shown in figure 3. Epithelial cells are characterized by an apical junctional complex, comprising tight junctions (TJ), adherens junctions (AJ) and desmosomes (Farquhar and Palade, 1963). TJ and AJ have critical role in the development and physiology of vertebrate epithelial tissues. TJ control the barrier function of epithelia and
maintain cell polarity and AJ regulate cell-cell adhesion and morphogenesis (Gumbiner, 1996; Shin et al., 2006).

![Diagram](http://genome.ucsc.edu/)

**Figure 3. Location of PLEKHA7 gene on chromosome 11 and its anatomy**

The molecular architecture of TJ and AJ by and large has been clarified in recent years. The junctions comprise transmembrane proteins that are linked to cytoskeletal filaments through a cytoplasmic plaque that contains scaffolding, adaptor and signaling proteins (Mitic and Anderson, 1998; Perez-Moreno and Fuchs, 2006; Guillemot et al., 2008; Takai et al., 2008; Meng and Takeichi, 2009). A recent study showed that E-cadherin, a major transmembrane protein of AJ, associates with microtubules through a protein complex comprising p120 ctn and the newly identified proteins PLEKHA7 and Nezha (Meng et al., 2008).
2.3.2.1 Pleckstrin homology domain, family A, member 7 and hypertension:

PLEKHA7 has been implicated in heart development and hypertension. Single nucleotide polymorphisms of PLEKHA7 locus are known to be associated with diastolic high blood pressure in genome-wide studies on Caucasian and Asian ethnic groups (Levy et al., 2009; Hong et al., 2010; Lin et al., 2011). The cellular mechanisms whereby mutations in the PLEKHA7 locus lead to increased blood pressure are unknown. Knockdown studies show that the zebrafish homolog of PLEKHA7 known as Hadp1, is required for cardiac contractility and morphogenesis, through a mechanism involving the regulation of intracellular Ca\(^{2+}\) handling (Wythe et al., 2011). Two large-scale genome-wide association studies (GWAs) show that variants in PLEKHA7 were significantly related to hypertension (Odds ratio [OR] and (95% confidence interval [CI]): 1.19 (1.01–1.41) in logistic regression analyses after adjusted by age, sex and BMI (Lin et al., 2011).

2.3.3 Unc-51-like kinase (ULK4):

Protein kinases have been known to play crucial roles in cellular regulation, including cell growth, differentiation, metabolism and gene expression in the eukaryotic cells. An increasing number of protein kinase genes are being identified in the course of genome sequencing projects of various eukaryotes and to date protein kinases constitute one of the largest gene superfamilies (Meharena et al., 2013). ULK4 codes for serine-threonine protein
kinase which is found to have possible role in many signal transduction reactions.

Figure 4. Location of ULK4 gene on chromosome 3 and its anatomy

Autophagy is a dynamic and highly regulated process of self-digestion. In eukaryotic cells autophagy occurs constitutively at low levels to perform housekeeping functions such as destruction of dysfunctional organelles. Upregulation occurs in the presence of external stressors (e.g. starvation, hormonal imbalance, oxidative stress) and internal needs (e.g. removal of protein aggregates) suggesting that the process is an important survival mechanism. It plays an adaptive role to protect organisms against diverse pathologies (Mizushima et al., 2008), including heart disease (Martinet et al., 2007). Under
rare circumstances the self-cannibalistic function of autophagy may be deleterious (Levine and Kroemer, 2008). Various evidences indicate that autophagy is associated with heart disease, cancer and a number of neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's disease. Autophagy also plays a role in development, aging and immunity (Delgado et al., 2008; Winslow and Rubinsztein, 2008; Lunemann and Munz, 2009).

To date, more than 30 ATG (autophagy-related) genes involved in autophagy have been identified in yeast and many of these are functionally conserved in higher eukaryotes (Suzuki and Ohsumi, 2009). Unc-51-like kinase 1 (ULK1), ULK2, ULK3 and ULK4 are the mammalian homologues of yeast Atg1 (Yan et al., 1998; Chan and Tooze, 2009). The ULK4 gene is conserved in many species including chimpanzee, dog, cow, mouse, rat, chicken, zebrafish, A.thaliana and rice (Krupnova et al., 2009). ULK4 has been implicated as a gene involved in the development of hydrocephalus (Vogel et al., 2011).

2.3.3.1 Unc-51-like kinase and hypertension:

Very little information about the role of this gene in the etiology of blood pressure is available. Autophagy has emerged as a significant contributor of hypertension and cardiovascular diseases (Wang et al., 2010). During hypertension or myocardial infarction, the heart undergoes a compensatory hypertrophic growth response. In such conditions, loss of autophagy in heart triggers cardiac dysfunction eventually leading to heart failure. Thus, the
involvement of ULK4 in the process of autophagy could vaguely explain its involvement in hypertension. Three linked nonsynonymous SNPs in this gene have been shown to be associated with diastolic blood pressure (Levy et al., 2009).

2.3.4 Cadherin 13 (CDH13):

Cadherins are a large family of transmembrane adhesion molecules which normally mediate calcium-dependent homophilic intercellular adhesion (Angst et al., 2001). They are directly involved in a wide variety of processes such as cell adhesion, cell sorting, cell survival, morphogenesis, formation of intercellular junctions, maintenance of tissue integrity and tumourigenesis (Rowlands et al., 2000). Calcium binding is essential for adherence function of cadherins, hence the name calcium dependent adherent protein.

T-cadherin (T-cad) or truncated cadherin is a unique atypical cadherin, because it lacks transmembrane and cytoplasmic domains and is attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor. Though T-cad is expressed in various organs and tissues, expression is predominant in cardiovascular tissues such as heart, aorta, carotid, iliac and renal arteries (Ivanov et al., 2001). Potential roles of this molecule include negative guidance of projecting axons in the embryonic nervous system, stimulation of angiogenesis and regulation of cell growth and migration (Philippova et al., 2009).

Human T-cad gene CDH13, is localized on chromosome 16q23.3 (Lee, 1996) along with other cadherins such as VE-cadherin, E-cadherin, P-cadherin,
CDH8 and CDH11. Earlier the T-cad gene was reported to have 1,169,627 base pairs (bps) comprising of 14 exons. It yields a transcript of 3711 bps encoding cDNA of 2142 bps that is translated to a GPI-anchored preproprotein of 714 amino acids (Ranscht and Dours-Zimmermann, 1991).

Figure 5. Location of CDH13 gene on chromosome 16 and its anatomy

According to the reports obtained recently from the databases the CDH13 gene consists of a transcript of 2,722 bp length possessing 15 exons, known to code for 760 amino acid residues (http://asia.ensembl.org/) (Figure 5). Mature T-cad protein has five extracellular cadherin domains (EC1- EC5).

2.3.4.1 T-Cadherin and angiogenesis:

T-cad is known to affect angiogenic behavior of endothelial cells bidirectionally. The up-regulation and downregulation of T-cad is found to be
proangiogenic (Philippova et al., 2006; Ghosh et al., 2007) and anti-angiogenic (Hebbard et al., 2008) respectively. Angiogenesis by T-cad is influenced by hemophilic binding between the cells which reduces adhesion and increases migration which ultimately leads to the proliferation of endothelial cells. T-cad is found to be upregulated in several pathological conditions including atherosclerosis (Ivanov et al., 2001), restenosis after balloon angioplasty (Kudrjashova et al., 2002) and tumor angiogenesis (Wyder et al., 2000).

### 2.3.4.2 T-Cadherin a receptor to adiponectin:

Adiponectin (APN) is an adipocyte derived hormone with levels ranging from 5 - 30µg/ml in healthy individuals. Reduced plasma adiponectin levels were observed in patients with obesity (Weyer et al., 2001), type 2 diabetes mellitus (Kondo et al., 2002), hypertension (Iwashima et al., 2004), myocardial infarction (Pischon et al., 2004), ischemic stroke (Chen et al., 2005) and coronary artery disease (Yang and Chuang, 2006). T-cadherin produced by cardiomyocytes is necessary for sequestering APN to heart. Experiments performed by Denzel et al. (2010) reported that ablation of T-cadherin expression dramatically increased levels of APN in the circulation. The excessive amount of APN was unable to associate with cardiac tissue and activate APN-dependent cardiac AMPK signaling, a major pathway associated with cardioprotective functions.

On the other hand, APN deficiency suppressed T-cadherin expression which is evident from the fact that APN-KO mice exhibited low cardiac T-cadherin protein expression. Cardiac phenotype was rescued by restoring T-cadherin expression after the administration of recombinant APN to APN-KO mice. This association reactivated AMPK signaling and controlled cardiac injury.
Absence of T-cad failed to rescue the phenotype. Thus, T-cadherin expressed on the surface of cardiomyocytes is necessary for the binding of APN and thereby facilitating cardioprotective functions.

Recently, GWAS for genetic markers in determining plasma adiponectin value in Korean population reported that genetic variants in \textit{CDH13} gene (coding T-cadherin), but not genetic variants in the \textit{ADIPOQ} gene (coding for adiponectin), influence adiponectin levels in Korean adults (Jee \textit{et al}., 2010). The QTL on \textit{CDH13} gene was also reported in Filipinos (Wu \textit{et al}., 2010) and more recently in a Chinese population (Chung \textit{et al}., 2011).

\subsection*{2.3.4.3 T-Cadherin and hypertension:}

GWAS performed in two European (Org \textit{et al}., 2009), and African-American populations (Adeyemo \textit{et al}., 2009) has exposed a number of polymorphic markers in \textit{CDH13} gene showing associations with EH. Kidambi \textit{et al}., 2012, replicated the study conducted by Adeyemo \textit{et al}, 2009 in an African-American population from the mid-western United States. Borderline associations were observed for two SNPs viz., \textit{rs7200009} and \textit{rs17177428} of \textit{CDH13} gene. The former was found to be associated with systolic and diastolic BP, whereas the latter was associated with diastolic BP alone. Taken together, the information acquired from the GWAS and other functional studies denote \textit{CDH13} a potential gene for further analysis with respect to the hypertension phenotype.
2.3.5 Lymphocyte-specific adaptor protein (SH2B3):

Inflammation and hypertension are linked and this has been proved by the presence of circulating inflammatory molecules such as C-Reactive Protein (CRP) and Interleukin-6 (IL-6) in hypertensive patients (Pauletto and Rattazzi, 2006). Extracellular signals relayed from the plasma membrane to specific intracellular sites are a key step of cellular regulation leading to inflammation. Cellular responses to external intrinsic signals are coordinated through specific protein–protein and protein–phospholipid interactions mediated by “adaptor proteins”. Adaptors have multiple functions such as determining the localization of signaling proteins in the cell, coordinating the signals involved in cell activation and bringing together the enzymes and substrates that drive the activation process (Figure 6). Expression of these adaptor proteins is either ubiquitous or restricted to selected cell types, where they play a specialized role by controlling differentiation and function (Devalliere and Charreau, 2011).

Figure 6. SH2B3 adaptor functions and outcome of mutation in the gene.
Lnk (SH2B3) is a member of the SH2B family of adaptor proteins which are implicated in integration and regulation of multiple signaling events. The SH2-B (Src homology 2-B) protein family contains SH2B1 and SH2B2, originally named SH2-B and APS (adaptor protein with PH and SH2 domains), respectively. Lnk is structurally composed of a number of functional domains: a carboxyl-terminal Src homology 2 (SH2) domain, which is essential for specific binding to phosphotyrosine residue, a pleckstrin homology (PH) domain, which recognize phosphoinositides and control protein translocation to the cell membrane, proline-rich regions, dimerization domain (DD) and several putative tyrosine phosphorylation motifs (Maures et al., 2007). The chromosomal location and the SH2B3 gene anatomy is shown in figure 7.

Figure 7. Location of SH2B3 gene on chromosome 12 and its anatomy
Lnk has been shown to negatively control receptor activation such as stem cell factor (SCF) receptor (Simon et al., 2008), thrombopoietin receptor (MPL) (Seita et al., 2007) erythropoietin receptor (EPOR) (Tong et al., 2005), platelet-derived growth factor receptor (PDGFR) (Gueller et al., 2011) and macrophage colony-stimulating factor receptor (c-Fms) (Gueller et al., 2010).

![Schematic representation of the structural organization of the Lnk (SH2B3) adaptor protein. PH: Pleckstrin homology domain, SH2: Src homology 2 domain, Tyr: tyrosine sites.](image)

2.3.5.1 Lymphocyte-specific adaptor protein and disease associations:

Functional investigation of the effect of the SH2B3 genotype in response to lipopolysaccharide and muramyl dipeptide revealed that carriers of the SH2B3 rs3184504 risk allele showed stronger activation of the NOD2 recognition pathway. This suggests that SH2B3 plays a role in protection against bacterial infection (Zhernakova et al., 2010). Recently, genetic studies reported a role for Lnk gene polymorphism and mutations in various diseases including type 1 diabetes (T1D) (Reddy et al., 2011), hypertension (Levy et al., 2009),
myocardial infarction (Gudbjartsson et al., 2009), celiac disease (Hunt et al., 2008; Smyth et al., 2008), myeloproliferative diseases (Oh et al., 2010), erythrocytosis (Lasho et al., 2010), systemic lupus erythematosus (Gateva et al., 2009), rheumatoid arthritis (Coenen et al., 2009) and multiple sclerosis (Alcina et al., 2010).

2.3.5.2 **Lymphocyte-specific adapter protein and hypertension:**

Experimental evidences suggest that there is a link between hypertension and inflammation (Pauletto and Rattazzi, 2006). It involves complex interplay between systemic inflammation, vascular cells activation and structural changes in the arteries. *SH2B3* gene, mapped to the locus 12q24 was recently found to be associated with coronary heart disease and hypertension. The SNP rs3184504 in *SH2B3* is one of the blood pressure SNPs determining a risk allele for both systolic and diastolic blood pressure (Levy et al., 2009). The SNP rs3184504, in exon 3 is a missense variant (R262W; 784 T>C) that introduces an amino acid substitution (arginine to tryptophan) in the PH domain involved in plasma membrane targeting (Li et al., 2000). These genetic variations can affect protein function by altering gene expression and protein levels or by altering the structure of the encoded protein.

The rs3184504 T allele, associated with increased blood pressure is known to cause increased cytokine production (Zhernakova et al., 2010). The SNP impacts blood pressure through an action specific to cells outside of the immune system which supports the hypothesis for a role of Lnk in vascular biology and homeostasis. In addition, the involvement of this SNP with a panel
of autoimmune diseases may also suggest that immune response pathways may influence blood pressure by mechanisms not yet clearly defined.

2.3.6 Solute carrier family 6, member 9 (SLC6A9):

Solute carriers are membrane proteins that control the uptake and efflux of various solutes, including amino acids, sugars and drugs (Hediger et al., 2004). The Human Gene Nomenclature Committee (HGNC) of the Human Genome Organization (HUGO) has classified ~400 human solute carriers into 47 families (Povey et al., 2001). The SLC6 family of proteins has 20 members in the human genome. It acts as specific transporters for neurotransmitters, amino acids and osmolytes (Chen et al., 2004).

SLC6 transporters can be divided in 4 subgroups based on the substrate they translocate. The neurotransmitter transporters (NTT) which include 3 γ-aminobutyric acid (GABA) transporters (GAT), 2 glycine transporters (GLY) and the monoamine (dopamine (DAT), serotonin (SERT) and norepinephrine (NET) transporters; the amino acid transporters which include proline (PROT, IMINO), cationic and neutral amino acid transporters (AA0, AA0,+) ; the osmolyte transporters which include the betaine (BGT1), taurine (TauT) transporters and creatine transporters (CT) and 1 orphan transporter (Amara and Kuhar, 1993).

In humans, the SLC6 family of transporters is one of the most clinically relevant protein groups with links to orthostatic intolerance, attention deficit
hyperactivity disorder (ADHD) (Mazei-Robison et al., 2008), addiction, osmotic imbalance, X-linked mental retardation (Martinez-Munoz et al., 2008), Hartnup disorder, hyperekplexia, Tourette syndrome, schizophrenia, Parkinson disease (PD), autism and mood disorders such as depression, anxiety, obsessive compulsive disorder (OCD) and post-traumatic stress disorder (PTSD) (Hahn and Blakely, 2007). SLC6A9, a member of this family encodes Na\(^+\) and Cl\(^-\) dependent glycine transporter which is involved in the inhibitory glycinenergic transmission.

![Diagram of SLC6A9 gene on chromosome 1]

**Figure 10. Location of SLC6A9 gene on chromosome 1 and its anatomy**

Human SLC6A9 gene has been mapped to chromosome band 1p33 by *in situ* hybridization (Jones et al., 1995). Two different high affinity plasma membrane transporters GlyT1 (SLC6A9) and GlyT2 (SLC6A5) have been discovered so far
(Guastella et al., 1992; Liu et al., 1992). Both the transporters are encoded by different genes and multiple splice variants have also been described (Adams et al., 1995; Ebihara et al., 2004).

GlyT1 shows a broader expression pattern in the glial cells of the brain stem and spinal cord, the regions which are rich in glycinergic neurotransmission (Zafra et al., 1995). About half of the transporters in SLC6 family co-transport their substrates together with 2 Na\(^{2+}\) ions and one Cl\(^{-}\) ion. The number of Na\(^{2+}\) ions could vary from one to three, for eg., GlyT1 requires 2Na\(^{2+}\) and 1Cl\(^{-}\) per transport cycle whereas GlyT2 requires 3Na\(^{2+}\) and 1Cl\(^{-}\) for transport (Roux and Supplisson, 2000). Therefore, the accumulative power of GlyT1 is less than GlyT2.

2.3.6.1 Solute carrier family 6, member 9 and hypertension:

The sympathetic nervous system (SNS) plays a vital role in the regulation of arterial pressure. SNS hyperactivity has been implicated as a primary precursor of essential hypertension in both humans and animal models of the disease (Wyss, 1993). The principal activity of SLC6A9 protein is the termination of synaptic activity through the removal of neurotransmitters. GlyT1 maintains significant extracellular glycine concentration which is sufficient to co-activate NMDA (N-methyl D-aspartate) receptors through the glycine site. Any change in the concentration or membrane potential results in the removal of glycine thereby modulating glutamatergic neurotransmission. Thus, GlyT1 is essential for regulating glycine levels at synapses. In this context, SLC6A9 seems to be a
promising candidate gene for essential hypertension since they play an important role in maintaining sympathetic activity in the central nervous system (Ueno et al., 2009).

2.3.7 Mitofusin-2 (MFN2):

Mitochondria are cellular organelles which play a fundamental role in respiration, substrates oxidation, ATP production and apoptosis. In addition, it is also involved in essential biochemical pathways, amino acid synthesis, steroid metabolism, iron metabolism, fatty acid oxidation and calcium homeostasis (Santel, 2006). Dynamic changes are known to occur in the shape, localization and number of mitochondria even within a single eukaryotic cell.Mitochondrial remodeling is regulated by cell division and fusion in response to physiological and environmental signals (Chen and Chan, 2004). This process is vital to accommodate diverse demands on mitochondrial function in various cell types during growth, differentiation and maintenance (Cerveny et al., 2007). Equilibrium between fusion and fission of mitochondria should be maintained so as to ensure proper energy metabolism, oxidation, calcium signaling and apoptosis. Proteins which are involved in the control of mitochondrial fusion are mitofusin 1 and 2 found in the outer membrane and OPA1 located in the inner mitochondrial membrane. Mitochondrial fusion is a two step process which is carried out by combined action of MFN1 and MFN2 which brings about outer membrane fusion and OPA1 regulating the inner membrane fusion (Malka et al., 2005).
The association of *MFN2* with essential hypertension has been recorded in recent years. Mitofusin 2, is also known as hyperplasia suppressor gene (*HSG*) and mitochondrial assembly regulatory factor (MARF). It is composed of 757 residues (Koshiba *et al.*, 2004) and is highly expressed in the heart, but is downregulated in the heart in response to hypertrophic stimuli (Fang *et al.*, 2007). It belongs to the family of large GTP-binding proteins and located on the short (p) arm of chromosome 1 at position 36.22 (Huang *et al.*, 2011). The expression product of *MFN2/HSG* is completely dependent on its GTPase activity.
(Chen et al., 2003). It participates in mitochondrial fusion and contributes to the maintenance and operation of the mitochondrial network. Though mitofusin is described as a mitochondrial protein, it is also found in endoplasmic reticulum (ER), controlling ER morphology and its tethering to mitochondria (de Brito and Scorrano, 2008). Dysregulation of mitofusin 2 is shown to trigger vascular proliferative disorder in experimental animal models as well as in essential hypertensive patients (Chen et al., 2004; Liu et al., 2007).

### 2.3.7.1 Mitofusin 2 and hypertension:

Ras signaling is the central pathway for a wide array of cardiovascular diseases such as hypertensive proliferation, injury-associated arterial restenosis, cardiac hypertrophy and failure, angiogenesis and endothelial dysfunction. Checkpoints in Ras pathway have been the major focus of cardiovascular biology and medicine (Chien and Hoshijima, 2004). *MFN2* being an endogenous Ras inhibitor suppressed VSMC proliferation by inhibiting Ras-Raf-ERK1/2 pathways (Chen et al., 2003). Any deregulation of *MFN2* expression has been linked to vascular proliferative disorders such as hypertension, atherosclerosis and restenosis after vascular injury (Guo et al., 2007). Since, EH is a disease characterized by hyperplasia of VSMCs, *MFN2* can be considered as a potential therapeutic target for EH and other cardiovascular disorders. Reduced expression of *MFN2* has also been reported in obese (Bash et al., 2003) and diabetic subjects (Bash et al., 2005). Recently, two studies in Chinese population (Wang et al., 2011; 2013) have identified novel variants exhibiting strong association with essential hypertension.
2.4 PHARMACOGENOMICS:

Management of blood pressure is essential for prevention of complications due to essential hypertension. Anti-hypertensive drugs currently in use are diuretics, beta-blockers, angiotensin converting enzyme inhibitors, calcium channel blockers and angiotensin receptor blockers (Minushkina et al., 2005). Despite the plethora of treatment options, the blood pressure control rates are less than 50%. This fact clearly establishes the inability to choose the antihypertensive drug likely to be most effective for an individual patient (Johnson, 2012). Inter individual variation in terms of genetic polymorphisms has been found to underlie pathophysiology of diseases which can also affect the efficacy of therapy.

The goal of hypertension pharmacogenomics relies on the genetic information along with clinical and demographic data to select antihypertensive regimen which is likely to provide greatest efficacy with minimal risk of adverse effects. For example: blacks respond slightly better than whites to diuretics and calcium channel blockers, whereas whites respond slightly better than blacks to ACE inhibitors and β-blockers (Brewster, 2004). This data shows that different pathways cause hypertension in different ethnic groups. Many of the pharmacogenetics studies have focused on single nucleotide polymorphisms (SNPs) within genes involved in the pathophysiology of EH. Polymorphic genes that encode elements of metabolism, absorption, transport, elimination of drugs and receptor systems are considered to be main candidates for pharmacogenetic studies. Personalized therapy targeted towards the specific pathway based on the
genetic profile and ethnicity may improve drug response thereby decreasing the global burden of EH. Additionally, whole genome mapping of hypertension and blood pressure traits coupled with the understanding of pharmacogenetics and pharmacokinetics of current drugs will enable the discovery of new drug targets in future.