Essential hypertension is complex disease affecting a large proportion of adult population. The scientific and technical resources available have enabled researchers to systematically pursue the identification of gene variation that causes heritable susceptibility to hypertension. Until the advent of two large scale meta-analyses projects of genome wide association studies (GWAS) from the Global BP Genetics (Global BPgen) and Cohorts for Heart and Ageing Research in Genome Epidemiology (CHARGE) consortia, the genetic analysis on the determinants of hypertension yielded only a limited success. Although these studies were carried out exclusively in the populations of European decent, the results represent an important advancement in the hypertension genome research (Dominiczak and Munroe, 2010). Recently, the Asian Genetic Epidemiology Network BP (AGEN-BP) was established to identify genetic variants influencing BP among populations of East-Asian ancestry (Kato et al., 2011).

Several genomic surveys have already revealed a substantial divergence in allele frequency (Adeyemo and Rotimi, 2010), linkage disequilibrium and haplotype structure (Evans and Cardon, 2005; Conrad et al., 2006) across populations. Both genetic and environmental influences contribute to the heterogeneity of complex traits. In this context, studies in other populations or ethnic groups will not only provide a clue on the effect of these putative genetic variants but also facilitate the identification of novel variants with more pronounced effect on BP regulation.

Since blood pressure is maintained by a complex integrated network of systems encompassing renal, neuronal, endocrine and vascular mechanisms, it is
a herculean task to attribute BP variation to a small subset of genes. Thus, in the present study, eleven markers from seven different genes viz., rs2681492, rs2681472 of ATP2B1, rs11024074 of PLEKHA7, rs1052501 of ULK4, rs7200009, rs11860907, rs16960421 and rs17177428 of CDH13, rs3184504 of SH2B3, rs2286245 of SLC6A9 and rs1474868 of MFN2 which are directly or indirectly involved in BP regulation have been selected based on their GWAS score to analyze their role in the susceptibility to essential hypertension in south Indian population. To our knowledge, this study provides the first report about the selected SNPs in hypertensives representing south Indian population.

**ATP2B1 (plasma membrane calcium ATPase) gene polymorphisms:**

ATP2B1 encodes the plasma membrane calcium ATPase isoform 1, which plays a critical role in intracellular calcium homeostasis. In addition, it is also suggested that ATP2B1 plays a major role in vascular smooth muscle contraction. Ca\(^{2+}\) ATPase represents most important calcium ejection system in the VSMC (Brading and Lategan, 1985). Increased Ca\(^{2+}\) influx in VSMC may contribute to increased contractility and promote rise in blood pressure (van Breemen et al., 1986, 1987). Genetic alterations may affect the activity or affinity of the isoform contributing to a hypertensive state (Benkwitz et al., 1999). Mice with VSMC-specific knockout (KO) of ATP2B1 were generated to test the relationship between ATP2B1 and hypertension. The KO mice expressed significantly lower levels of ATP2B1 mRNA and protein in the aorta when compared to controls. Subsequently, a higher systolic blood pressure was also recorded in the KO mice.
The cultured VSMC of KO mice showed increased intracellular calcium concentration (Kobayashi et al., 2012).

Genetic variants of ATP2B1 gene have been established as one of the strong markers associated with hypertension. Recent GWAS have revealed their positive association in European, Japanese, Korean populations and negative association in a Chinese population. Six individual cohorts (AGES, n=3,219; ARIC, n=8,047; CHS, n=3,277; FHS, n=8,096; RS, n=4,737; RES, n=1760) combined (CHARGE consortium) to perform a GWAS to investigate the association of SNP markers with EH. Among the top ten loci analyzed in a European population by CHARGE consortia, eight loci attained a genome wide significance (P < 5 x 10^{-8}). The ATP2B1 markers demonstrated a significant association with all three traits involving SBP, DBP and hypertension. The rs2681472 marker showed a strong association with diastolic blood pressure and hypertension (Levy et al., 2009).

A meta-analysis involving 15,909 cases and 18,529 controls was performed to assess the association rs2681472 marker of ATP2B1 gene with hypertension risk in East Asians. The results obtained showed a significant association (OR = 1.18; 95% CI: 1.10-1.27; P = 0.000) with hypertension susceptibility (Xi et al., 2012).

The Amagasaki cohort, Fukuoka cohort and the Kita-Nagoya Genomic Epidemiology [KING] study cohort performed a meta-analysis in a randomly selected Japanese general population to evaluate the effect of seven genetic variants on blood pressure and hypertension. One among them was rs2681472
marker of \textit{ATP2B1} gene which showed a significant association with hypertension (OR = 1.10; 95\% CI: 1.04 – 1.17; \( P = 0.002 \)) (Takeuchi \textit{et al.}, 2010).

A cross-sectional study on the She ethnic minority of China, ranging from 20–80 years of age, indicated a significantly higher minor allele frequency for \textit{rs2681472} marker in prehypertensive and hypertensive groups when compared to normotensive subjects. Despite this fact, the marker had no association with both BP traits and hypertension in She population after adjusting for age, sex and BMI (Lin \textit{et al.}, 2011). A recent study on women of Northern Han Chinese population, has reported that the SNP marker \textit{rs2681472} has been associated with early onset preeclampsia, a condition characterized by hypertension and proteinuria (Wan \textit{et al.}, 2014). The present study indicates that there is no significant association between cases and controls for \textit{rs2681492} depicting A/G polymorphism of \textit{ATP2B1} gene.

The TT homozygous genotype of \textit{rs2681472} polymorphism (\textit{ATP2B1} gene) acts as an independent risk factor for EH which is evident from the unadjusted odds ratio (p value = 0.051, OR = 1.257). On adjusting for confounding factor, BMI, the p value shows a marginal significance (p = 0.071, OR = 1.237). These results indicate that individuals with TT homozygous genotype are 1.2 times more susceptible to EH when compared to individuals with CC or CT genotypes. The additive model also showed a significant difference between C and T allele with a p value of 0.038 (OR = 1.213). These results suggest that the TT homozygous genotype and T allele acts as a risk
factor for essential hypertension. Categorization of study subjects based on gender, revealed that females exhibited a similar association with essential hypertension, whereas males did not, indicating that rs2681472 marker of ATP2B1 gene is more significantly associated with hypertension in female than in male. Since the distribution of ATP2B1 gene polymorphisms were in concordance with Hardy-Weinberg equilibrium, the results of this study are unlikely to be biased by population stratification or admixture for essential hypertension.

The Japanese Millenium Genome project has identified rs2681472 as a significant marker associated with hypertension in both screening and replication panels. Several other SNPs of ATP2B1 gene such as rs2070759 and rs11105378 have also shown strong association with hypertension (Tabara et al., 2010). A meta-analysis by two Korean cohorts, KARE (Korean Association REsource) and Health2 have confirmed that the marker rs17249754 in ATP2B1 gene increases risk to hypertension (Hong et al., 2010a; 2010b).

Genome wide microsatellite scan was employed to identify the susceptible locus for EH. The study group comprised of a single large family of 22 individuals from Shanghai, China. Of the 22 family members 11 were diagnosed with hypertension. Interestingly, some of them were diagnosed with early-onset hypertension earlier than 20 years of age. On analyzing 424 polymorphic markers, the susceptible locus linked to hypertension phenotype was located in the 12q23.1 - q23.3 region. ATP2B1 was one of the candidate gene screened
Discussion

among 16 candidate genes in this region. Two SNPs in this gene (rs2681492 and rs2681472) were also genotyped. The results showed that these two SNPs were not associated with hypertension phenotype in the family studied. A major limitation of this study was that the intronic and intergenic regions were not investigated. So, the hypertension susceptibility loci might reside in any one of the intergenic or intronic region in the candidate genes analyzed (Dong et al., 2013).

Although the polymorphisms analyzed showed contradictory results in different ethnic groups, majority of the data support the fact that ATP2B1 plays a pivotal role in blood pressure regulation and hypertension etiology, thereby emphasizing the significance of carrying out further investigations to clarify the functional basis of the associations.

**PLEKHA7 (pleckstrin homology domain, family A, member 7) gene polymorphism:**

PLEKHA7 gene encodes pleckstrin homology domain-containing protein, family A member 7. This protein was reported to be involved in maintaining integrity of zona adherens, an epithelial cadherin-based cell–cell junction (Meng et al., 2008). Though it is difficult to determine the function of PLEKHA7 on the regulation of blood pressure, it is found to promote the incorporation of cadherin clusters, including E-cadherin and p120-catenin, into the higher-order structure
Discussion

of the zonula adherens which plays a vital role in cell signaling (Pulimeno et al., 2010).

GWAS involving the CHARGE consortia and Global BPgen have together identified two markers of *PLEKHA7* gene, *rs381815* and *rs11024074* which showed positive association with SBP and DBP respectively. The marker *rs11024074* produced a significant association (*P* = 2.8 X 10^-7) with diastolic BP (Levy et al., 2009). The results of the present study for screening of *rs11024074* of *PLEKHA7* gene indicate that the genotype distribution did not differ significantly between hypertensive patients and normotensive subjects.

Genetic variation of *rs381815* in *PLEKHA7* was associated with essential hypertension in She ethnic minority of China. Subjects with risk allele T of *PLEKHA7* gene had higher risk of hypertension compared to subjects with allele C (*OR*=1.19; *P* = 0.046) (Lin et al., 2011). Community based cohort study including the rural community Ansung and the urban community Ansan of Korea revealed that individuals who had the minor allele (T) of *rs381815* showed significantly increased SBP, DBP and hypertension risk (Hong et al., 2010b).

**ULK4 (unc-51-like kinase 4) gene polymorphism:**

**ULK4** encodes serine/theronine kinase of unknown function. The *rs1052501* marker of *ULK4* gene is a non-synonymous SNP marker leading to an amino acid change from alanine to threonine. The joint meta-analysis by CHARGE and Global BPgen showed suggestive evidence of association with
diastolic blood pressure (Levy et al., 2009). The results obtained from the present study did not show any significant difference in the genotype distribution amongst the cases and controls. No significant association was found between the marker rs1052501 of ULK4 gene in both male and female subjects before and after adjusting for confounding factors like age and BMI. Reports from other ethnic groups were not available to compare the results obtained from the present study.

**CDH13 (cadherin 13) gene polymorphisms:**

CDH13 gene codes for an adhesion glycoprotein T-cadherin that is a regulator of vascular wall remodeling and angiogenesis (Rubina et al., 2007). Abundant expression of T-cadherin in the myocardium and its association with cholesterol rich membrane domains of the cardiac sarcolemma has raised interest in analyzing their genetic background in relation to blood pressure regulation (Doyle et al., 1998). T-cadherin is also identified as a receptor for adiponectin (APN), a hormone with beneficial metabolic and cardiovascular properties (Shibata et al., 2009). Studies have shown that low APN serum levels correlate with development of cerebrovascular disease (Chen et al., 2005), coronary artery disease (Kumada et al., 2003), myocardial infarction (Hong et al., 2004), hypertension (Iwashima et al., 2004), left ventricular hypertrophy and other cardiovascular dysfunctions. All these features indicate this gene as a promising candidate for blood pressure regulation. Multiple SNP markers of CDH13
(cadherin 13) gene has been found to be in strong association with essential hypertension in European and African-American population (Adeyemo et al., 2009; Org et al., 2009; Kidambi et al., 2012). Kooperative Gesundheitsforschung in der Region Augsburg (KORA) S3 cohort performed a GWAS in the general population recruited from Southern Germany. The study showed significant association of CDH13 gene with all three BP traits: SBP, DBP and hypertension. The results were further replicated in two other European population based cohorts: KORA S4 (Germans) and HYPEST (Estonians) (Org et al., 2009).

The SNP markers rs7200009 (P = 1.08 X 10^{-3}) and rs11860907 (P = 5.71 X 10^{-4}) showed a significant association with systolic blood pressure, whereas markers rs16960421 (P = 1.82 X 10^{-3}) and rs17177428 (P = 3.42 X 10^{-3}) produced a significant association with diastolic blood pressure in African American population (Adeyemo et al., 2009). Kidambi et al, (2012) performed a replication analysis on the SNPs identified earlier by Adeyemo et al. (2009). The study included an independent population of African American subjects (n=2474) from the mid-western United States. Although the four markers, rs7200009, rs11860907, rs16960421 and rs17177428 of CDH13 gene showed no association with blood pressure, borderline associations were identified without statistical adjustments for multiple comparisons. SNP rs7200009 was associated with both systolic and diastolic blood pressure (P = 0.04) and SNP rs17177428 was associated with systolic blood pressure (P = 0.04).
This result is in compliance with the result obtained from the present study, wherein the marker rs7200009 showed a marginal significance (P = 0.088) between the genotype frequencies of the case and control groups. The TT genotype was found to be the high risk genotype which is evident from the recessive model comparison of TT vs CT+CC between case and control groups (P = 0.029; OR = 1.447). The significance remained the same after adjusting for BMI (P = 0.037; OR = 1.427). The additive model which compared the C vs T allele frequencies between case and control groups revealed the fact that C allele was protective with an odds ratio of 0.822 (p value = 0.041). A significant association was also found for the TT genotype after adjusting for BMI (P = 0.041) in female subjects. Whereas, the significance declined after adjusting for BMI in female subjects (P = 0.061). Thus the TT genotype carries risk in female subjects while no such association was observed in the male subjects.

The other marker of CDH13 gene rs17177428, showed a significant difference between the control and case groups (P = 0.049) in the dominant model. The GG homozygous genotype was found to be the risk genotype with an odds ratio of 1.415. But, the significance decreased on adjusting BMI (p = 0.083). Gender wise distribution did not show any significant association between hypertensive and normotensive groups.

The genetic analysis of the SNP marker rs16960421 of CDH13 revealed the presence of only two genotypes prevalent in the study group. HapMap analysis has revealed that T allele frequency was maximum in African ethnic groups followed by European and Americans. The T allele was almost absent in
the Asian ethnic groups as evident from the thousand genomes project (http://browser.1000genomes.org/). Historical migration has shaped the global distribution of alleles. Migration of individuals from the African ancestry to several parts of the world has contributed to the vast genetic diversity. In this context, the European, American and Asian ethnic groups could have gradually acquired C allele overtime and this could be the reason for complete absence or minimal presence of T allele in these populations. In addition, the T allele could have been deleterious in some way that the natural selection process has wiped out the allele from descendent populations.

The fourth marker of CDH13 gene rs11860907, did not produce any significant difference between the two study groups. Gender-wise analysis did not produce any significant association between case and control groups of male and female subjects. A crucial outcome of the study on three markers of CDH13 gene (rs7200009, rs17177428 and rs11860907) revealed a significant deviation from Hardy-Weinberg equilibrium in both control and case groups.

Over a period of time mutations do arise in an individual (founder) in a population. Immigration or emigration of these founder individuals may result in genetic drift, due to inbreeding or assortative mating. Genetic drift is common in smaller population and decreases with increase in population size (Zhang and Tier, 2009). Natural selection also modifies the probabilities that alleles are found in either homozygous or heterozygous form adds to the facts responsible for the deviation.
Discussion

Structure of the population is also vital in determining allele frequencies. It can result in spurious associations or marked deviations in allele frequency of particular loci. Population stratification is the presence of different allele frequencies among several subpopulations due to different ancestry (Grover et al., 2010). The interbreeding between two or more previously isolated population is known as admixture. The present day Indian population are the admixture of both ancestral north Indians and ancestral south Indians (Tamang et al., 2012). This recent ethnic admixture may also contribute to departure in HWE.

**SH2B3 (lymphocyte specific adaptor protein) polymorphism:**

*SH2B3* gene has a wide range of clinical significance. It has been shown to be strongly associated with essential hypertension (Newton-Cheh et al., 2009), celiac disease (Hunt et al., 2008), type I diabetes mellitus (Todd et al., 2007) and other autoimmune diseases (Gudbjartsson et al., 2009). It encodes Lnk, an adaptor protein that mediates the interaction between extra-cellular receptors, such as the T-cell receptor, the thrombopoietin receptor and intracellular signaling pathways. The SNP rs3184504 is a nonsynonymous SNP in exon 3 of *SH2B3* gene, leading to R262W (arginine to tryptophan) change in the pleckstrin homology domain. Rippati et al. (2010), carried out case-control analysis and prospective cohort study including subjects from Finland and Sweden. The reports suggest that the marker rs3184504 of *SH2B3* gene was associated with cardiovascular disease (OR = 1.10; P = 0.011) and myocardial infarction (OR = 1.15; P = 0.012).
Meta-analysis by the CHARGE and Global BPgen consortium have attained a genome wide significance for \textit{SH2B3} (\textit{rs3184504}) with systolic blood pressure (P = $4.5 \times 10^{-9}$) (Levy \textit{et al.}, 2009). Another report on GWAS in African American population also showed association with EH (P = 0.009) (Fox \textit{et al.}, 2011). The T allele of \textit{rs3184504} correlates with high diastolic blood pressure and is common in HapMap CEU (frequency = 0.45), whereas absent in Hapmap YRI, JBT and CHB samples which is an evidence for recent positive selection. Positive selection of the T allele was also observed in four European and Saharawi population. When a genetic variation is under positive selection it increases in prevalence in a population. Climate, diet and pathogen load causes a selective pressure in populations world-wide resulting in global allele frequency variation (Zhernakova \textit{et al.}, 2010).

The minor allele T, leading to a missense mutation results in the loss of \textit{SH2B3} function. This report suggests that the minor allele arose with an intermediate frequency in European-derived populations, conferring selective advantage of immune response to infectious pathogens. Although enhancing \textit{SH2B3} activity might seem attractive to reduce risk for multiple diseases, the evidence for positive selection of an apparent loss-of-function allele and pleiotropic consequences suggest that enhancing \textit{SH2B3} activity could have unintended consequences (Newton-Cheh \textit{et al.}, 2009).

In the present study, the genotype frequency between cases and controls did not differ significantly (P = 0.130). Though there was no association in model based study for the overall genotype analysis, female subjects showed a
significant association with essential hypertension. In the dominant model, CC genotype was found to be the risk genotype with an adjusted p value of 0.030 (OR = 1.455). Hence the risk that is estimated is 1.4 times more in individuals with CC genotype when compared to the other two genotypes. However, the unadjusted p value showed only a marginal significance with a p value of 0.067 (OR = 1.366). The additive model for female subjects also showed that the C allele poses a risk on an individual’s blood pressure phenotype (p value = 0.023, OR = 1.377). No such association was observed in the male subjects.

Pharmacogenomics Responses of Antihypertensive Responses (PEAR) study was performed to investigate whether the loci/SNP associated with BP/hypertension are also associated with BP response to antihypertensive drugs. The PEAR participants were Caucasian (60%) and African American (40%) hypertensive individuals. Around 37 SNPs were analyzed for this purpose. The associations of these markers with BP response to atenolol and hydrochlorothiazide (HCZT) monotherapy were assessed in 768 hypertensive patients. The SNP marker rs3184504 of SH2B3 gene was also assessed. This marker showed opposite effect of association in African Americans in comparison to Caucasians. The C allele was linked with better BP reduction in Caucasians treated with HCZT, whereas a slight increase in BP was observed in African Americans. The variation in the drug response due to ethnic disparity could also be precipitated by other underlying factors involved in the blood pressure regulation (Gong et al., 2012).
Substantial evidences from GWAS in different populations have immensely contributed to the knowledge about the role of SH2B3 gene with EH. Being a non-synonymous SNP leading to Arg262Trp change, this marker has attracted attention for functional analysis. Both genetic and functional validation is warranted to reveal the effect of this marker on blood pressure regulation.

**SLC6A9 (solute carrier family 6, member 9) polymorphism:**

SLC6A9 gene encodes glycine transporter, whose principal activity is the termination of synaptic activity through the removal of neurotransmitters. Recent reports have revealed the fact that an early stage of essential hypertension is accompanied by sympathetic hyperactivation. A genetic case control study in a Japanese population also found marginal association between hypertensives and normotensives. The OR was estimated to be 1.26 (95% CI: 0.99 – 1.62; P = 0.06) after BMI and age adjustment (Ueno et al., 2009). The polymorphic marker of SLC6A9 gene (rs2286245) did not produce significant association with essential hypertension in the south Indian population studied. The genotype frequency was found to be roughly the same in both normotensives and hypertensive groups. Gender-wise analysis also did not show any significant difference between the case and control groups of the population studied. Though the SLC6A9 gene has a strong functional role in the etiology of essential hypertension, the outcome of the present study could not be compared with other populations due to lack of reports.
**Discussion**

**MFN2 (mitofusin-2) polymorphism:**

Mitofusin, belongs to a family of GTP-binding proteins which is an essential component of the mitochondrial machinery. Mitochondrial oxidative stress due to imbalances in mitochondrial fusion and fission contributes to decline in mitochondrial function. This can lead to a wide variety of pathologies including hypertension and atherosclerosis (Guo et al., 2007). A recent study in the Chinese population revealed a gender based association between rs1474868 of *MFN2* gene with EH. The study revealed significant difference between normotensive and hypertensive male population (Wang et al., 2011). Another study in Chinese population also revealed an A > G variation of 5’-non coding region viz., -1248 of *MFN2* gene to be associated with hypertension (Wang et al., 2013). The SNP marker of *MFN2* gene (rs1474868) did not show any significant difference between the case and control groups in south Indian population studied. The genotype frequency was almost the same for both the case and control data sets. Gender-based study did not produce any significant association with EH. Mitofusin, which is highly expressed in heart, could be a vital target in studying the genetic basis of hypertension. Although, this protein has a potential role in several cardiovascular pathologies, the genetic basis has not been investigated to a larger extent. Globally, the genetic association of the polymorphism in mitofusin gene with that of EH has not been well established in other populations/ethnic groups. Hence, a clear conclusion could not be obtained to ascertain the correlation of rs1474868 polymorphism with hypertension.
Impact of SNP markers on gene expression and regulation:

The thousand genomes project and other such ongoing projects are aimed to identify and predict the function of genetic variants spanning genome. The consequence of polymorphism or genetic variants across the human genome depends on the location of the variant. The present study involves three types of variants which includes eight intronic, two exonic and one 3’UTR variant. Although introns, the non-coding sequence of the gene have little functional significance, they may influence the level of gene expression by interfering with the splicing mechanisms. They can affect many splicing regulatory elements leading to aberrant splicing. Intrinsic splicing enhancers (ISE) and intronic splicing silencers (ISS) allow specific splice sites to be distinguished from many cryptic splice sites that have same signal sequences. Genetic variants may also introduce or erase splice sites. Intronic variants may be located ~50bp from the splice sites or deep in the introns (Pagani and Baralle, 2004).

Polymorphisms in the exon region could contribute to an altered phenotype and at rare circumstances cause deleterious effects. The exon variants may either be synonymous (no change in amino acid) or non-synonymous (amino acid is changed). The changes caused in the DNA sequence due to a polymorphic variant may either lead to the production of a non-functional protein, a truncated protein or a protein with altered activity when compared to the native protein. Apart from these mechanisms, an exon variant can cause a constitutively included exon to be skipped leading to an aberrant mRNA and subsequent loss of the translational product (Pagani and Baralle, 2004).
Variants in regulatory regions are predicted to play a vital role in disease susceptibility of common complex disorders. Recent evidences demonstrate the effect of 3’-UTR variants on gene expression patterns. They exert this effect by influencing mRNA stability and translation (Akhter et al., 2012). The cis-acting determinants in the 3’UTR to which proteins bind either stabilize or destabilize the mRNA. These determinants in the 3’UTR may also interact with other sequences within the same mRNA. Therefore, any variation in these cis-acting determinants may affect the expression of a particular gene (Misquitta et al., 2001). Mammalian miRNA (miRNA) have been shown to target endogenous mRNA through 3’-UTR and interfere with translational output (Lytle et al., 2007). Polymorphisms in microRNA binding sites have been shown to disrupt the ability of miRNAs to target genes resulting in differential mRNA and protein expression. Many of the polymorphisms studied in recent years encompass these three variants which are considered to be crucial in gene regulation and expression. The single nucleotide variants may produce pathogenic effect or remain silent without causing any damaging result in an individual harboring it.